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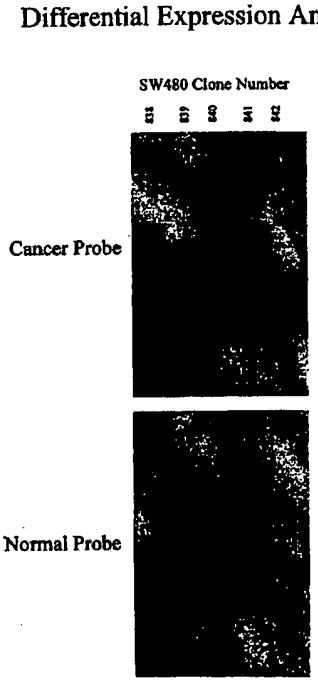
(57) Abstract

This invention relates to novel human genes, to proteins expressed by the genes, and to variants of the proteins. The invention also relates to diagnostic assays and therapeutic agents related to the genes and proteins, including probes, antisense constructs, and antibodies. The subject nucleic acids have been found to be differentially regulated in tumor cells, particularly colon cancer cell lines and/or tissue.



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NOVEL HUMAN GENES AND GENE EXPRESSION PRODUCTS

This application is based on Provisional Application No. 60/088,801, filed June 10, 1998, which is hereby incorporated herein by reference.

Field of the Invention

The present invention provides nucleic acid sequences and proteins encoded thereby, as well as probes derived from the nucleic acid sequences, antibodies directed to the encoded proteins, and diagnostic methods for detecting cancerous cells, especially colon cancer cells.

Background of the Invention

Colorectal carcinoma is a malignant neoplastic disease. There is a high incidence of colorectal carcinoma in the Western world, particularly in the United States. Tumors of this type often metastasize through lymphatic and vascular channels. Many patients with colorectal carcinoma eventually die from this disease. In fact, it is estimated that 62,000 persons in the United States alone die of colorectal carcinoma annually.

However, if diagnosed early, colon cancer may be treated effectively by surgical removal of the cancerous tissue. Colorectal cancers originate in the colorectal epithelium and typically are not extensively vascularized (and therefore not invasive) during the early stages of development. Colorectal cancer is thought to result from the clonal expansion of a single mutant cell in the epithelial lining of the colon or rectum. The transition to a highly vascularized, invasive and ultimately metastatic cancer which spreads throughout the body commonly takes ten years or longer. If the cancer is detected prior to invasion, surgical removal of the cancerous tissue is an effective cure. However, colorectal cancer is often detected only upon manifestation of clinical symptoms, such as pain and black tarry stool. Generally, such symptoms are present

only when the disease is well established, often after metastasis has occurred, and the prognosis for the patient is poor, even after surgical resection of the cancerous tissue. Early detection of colorectal cancer therefore is important in that detection may significantly reduce its morbidity.

Invasive diagnostic methods such as endoscopic examination allow for direct visual identification, removal, and biopsy of potentially cancerous growths such as polyps. Endoscopy is expensive, uncomfortable, inherently risky, and therefore not a practical tool for screening populations to identify those with colorectal cancer. Non-invasive analysis of stool samples for characteristics indicative of the presence of colorectal cancer or precancer is a preferred alternative for early diagnosis, but no known diagnostic method is available which reliably achieves this goal. A reliable, non-invasive, and accurate technique for diagnosing colon cancer at an early stage would help save many lives.

Summary of the Invention

The present invention provides nucleic acid sequences and proteins encoded thereby, as well as probes derived from the nucleic acid sequences, antibodies directed to the encoded proteins, and diagnostic methods for detecting cancerous cells, especially colon cancer cells.

In one aspect, the invention provides an isolated nucleic acid comprising a nucleotide sequence which hybridizes under stringent conditions to a sequence of SEQ ID Nos. 1-127 or a sequence complementary thereto. In a related embodiment, the nucleic acid is at least about 80% or about 100% identical to a sequence corresponding to at least about 12, at least about 15, at least about 25, or at least about 40 consecutive nucleotides up to the full length of one of SEQ ID Nos. 1-127 or a sequence complementary thereto or up to the full length of the gene of which said sequence is a fragment. In certain embodiments, a nucleic acid of the present invention includes at least about five, at least about ten, or at least about twenty nucleic acids from a region designated as novel in Table 2. In certain other embodiments, a nucleic acid of the present invention includes at least about five, at least about ten, or at least about twenty nucleotides which are not included in corresponding clones whose accession numbers are listed in Table 2.

In one embodiment, the invention provides a nucleic acid comprising a nucleotide sequence which hybridizes under stringent conditions to a sequence of SEQ ID Nos. 1-127 or a sequence complementary thereto, and a transcriptional regulatory sequence operably linked to the nucleotide sequence to render the nucleotide sequence suitable for use as an expression vector. In another embodiment, the nucleic acid may be included in an expression vector capable of replicating in a prokaryotic or eukaryotic cell. In a related embodiment, the invention provides a host cell transfected with the expression vector.

In another embodiment, the invention provides a transgenic animal having a transgene of a nucleic acid comprising a nucleotide sequence which hybridizes under stringent conditions to a sequence of SEQ ID Nos. 1-127 or a sequence complementary thereto incorporated in cells thereof. The transgene modifies the level of expression of the nucleic acid, the stability of an mRNA transcript of the nucleic acid, or the activity of the encoded product of the nucleic acid.

In yet another embodiment, the invention provides substantially pure nucleic acid which hybridizes under stringent conditions to a nucleic acid probe corresponding to at least about 12, at least about 15, at least about 25, or at least about 40 consecutive nucleotides up to the full length of one of SEQ ID Nos. 1-127 or a sequence complementary thereto or up to the full length of the gene of which said sequence is a fragment. The invention also provides an antisense oligonucleotide analog which hybridizes under stringent conditions to at least 12, at least 25, or at least 50 consecutive nucleotides of one of SEQ ID Nos. 1-850 up to the full length of one of SEQ ID Nos. 1-850 or a sequence complementary thereto or up to the full length of the gene of which said sequence is a fragment, and which is resistant to cleavage by a nuclease, preferably an endogenous endonuclease or exonuclease.

In another embodiment, the invention provides a probe/primer comprising a substantially purified oligonucleotide, said oligonucleotide containing a region of nucleotide sequence which hybridizes under stringent conditions to at least about 12, at least about 15, at least about 25, or at least about 40 consecutive nucleotides of sense or antisense sequence selected from SEQ ID Nos. 1-127 up to the full length of one of SEQ ID Nos. 1-127 or a sequence complementary thereto or up to the full length of the gene of which said sequence is a fragment. In preferred embodiments,

the probe selectively hybridizes with a target nucleic acid. In another embodiment, the probe may include a label group attached thereto and able to be detected. The label group may be selected from radioisotopes, fluorescent compounds, enzymes, and enzyme co-factors. The invention further provides arrays of at least about 10, at least about 25, at least about 50, or at least about 100 different probes as described above attached to a solid support.

In yet another embodiment, the invention pertains to a method of determining the phenotype of a cell, comprising detecting the differential expression, relative to a normal cell, of at least one nucleic acid which hybridizes under stringent conditions to one of SEQ ID Nos. 1-850, wherein the nucleic acid is differentially expressed by at least a factor of two, at least a factor of five, at least a factor of twenty, or at least a factor of fifty.

In another aspect, the invention provides polypeptides encoded by the subject nucleic acids. In one embodiment, the invention pertains to a polypeptide including an amino acid sequence encoded by a nucleic acid comprising a nucleotide sequence which hybridizes under stringent conditions to a sequence of SEQ ID Nos. 1-127 or a sequence complementary thereto, or a fragment comprising at least about 25, or at least about 40 amino acids thereof. Further provided are antibodies immunoreactive with these polypeptides.

In still another aspect, the invention provides diagnostic methods. In one embodiment, the invention pertains to a method for determining the phenotype of cells from a patient by providing a nucleic acid probe comprising a nucleotide sequence having at least 12, at least about 15, at least about 25, or at least about 40 consecutive nucleotides represented in a sequence of SEQ ID Nos. 1-850 up to the full length of one of SEQ ID Nos. 1-850 or a sequence complementary thereto or up to the full length of the gene of which said sequence is a fragment, obtaining a sample of cells from a patient, providing a second sample of cells substantially all of which are non-cancerous, contacting the nucleic acid probe under stringent conditions with mRNA of each of said first and second cell samples, and comparing (a) the amount of hybridization of the probe with mRNA of the first cell sample, with (b) the amount of hybridization of the probe with mRNA of the second cell sample, wherein a difference of at least a factor of two, at least a factor of five, at least a factor of twenty, or at least

a factor of fifty in the amount of hybridization with the mRNA of the first cell sample as compared to the amount of hybridization with the mRNA of the second cell sample is indicative of the phenotype of cells in the first cell sample. Determining the phenotype includes determining the genotype, as the term is used herein.

In another embodiment, the invention provides a test kit for identifying an transformed cells, comprising a probe/primer as described above, for measuring a level of a nucleic acid which hybridizes under stringent conditions to a nucleic acid of SEQ ID Nos. 1-850 in a sample of cells isolated from a patient. In certain embodiments, the kit may further include instructions for using the kit, solutions for suspending or fixing the cells, detectable tags or labels, solutions for rendering a nucleic acid susceptible to hybridization, solutions for lysing cells, or solutions for the purification of nucleic acids.

In another embodiment, the invention provides a method of determining the phenotype of a cell, comprising detecting the differential expression, relative to a normal cell, of at least one protein encoded by a nucleic acid which hybridizes under stringent conditions to one of SEQ ID Nos. 1-850, wherein the protein is differentially expressed by at least a factor of two, at least a factor of five, at least a factor of twenty, or at least a factor of fifty. In one embodiment, the level of the protein is detected in an immunoassay. The invention also pertains to a method for determining the presence or absence of a nucleic acid which hybridizes under stringent conditions to one of SEQ ID Nos. 1-127 in a cell, comprising contacting the cell with a probe as described above. The invention further provides a method for determining the presence of absence of a subject polypeptide encoded by a nucleic acid which hybridizes under stringent conditions to one of SEQ ID Nos. 1-127 in a cell, comprising contacting the cell with an antibody as described above. In yet another embodiment, the invention provides a method for determining the presence of an aberrant mutation (e.g., deletion, insertion, or substitution of nucleic acids) or aberrant methylation in a gene which hybridizes under stringent conditions to a sequence of SEQ ID Nos. 1-383 or a sequence complementary thereto, comprising collecting a sample of cells from a patient, isolating nucleic acid from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a nucleic acid sequence of SEQ ID Nos. 1-850 under conditions such that

hybridization and amplification of the nucleic acid occurs, and comparing the presence, absence, or size of an amplification product to the amplification product of a normal cell.

In one embodiment, the invention provides a test kit for identifying transformed cells, comprising an antibody specific for a protein encoded by a nucleic acid which hybridizes under stringent conditions to any one of SEQ Nos. 1-850. In certain embodiments, the kit further includes instructions for using the kit. In certain embodiments, the kit may further include instructions for using the kit, solutions for suspending or fixing the cells, detectable tags or labels, solutions for rendering a polypeptide susceptible to the binding of an antibody, solutions for lysing cells, or solutions for the purification of polypeptides.

In yet another aspect, the invention provides pharmaceutical compositions including the subject nucleic acids. In one embodiment, an agent which alters the level of expression in a cell of a nucleic acid which hybridizes under stringent conditions to one of SEQ ID Nos. 1-850 or a sequence complementary thereto is identified by providing a cell, treating the cell with a test agent, determining the level of expression in the cell of a nucleic acid which hybridizes under stringent conditions to one of SEQ ID Nos. 1-850 or a sequence complementary thereto, and comparing the level of expression of the nucleic acid in the treated cell with the level of expression of the nucleic acid in an untreated cell, wherein a change in the level of expression of the nucleic acid in the treated cell relative to the level of expression of the nucleic acid in the untreated cell is indicative of an agent which alters the level of expression of the nucleic acid in a cell. The invention further provides a pharmaceutical composition comprising an agent identified by this method. In another embodiment, the invention provides a pharmaceutical composition which includes a polypeptide encoded by a nucleic acid having a nucleotide sequence that hybridizes under stringent conditions to one of SEQ ID Nos. 1-850 or a sequence complementary thereto. In one embodiment, the invention pertains to a pharmaceutical composition comprising a nucleic acid including a sequence which hybridizes under stringent conditions to one of SEQ ID Nos. 1-850 or a sequence complementary thereto.

Brief Description of the Figure

The figure depicts an exemplary assay result for determining differential expression of gene products in cells.

Detailed Description of the Invention

The invention relates to nucleic acids having the disclosed nucleotide sequences (SEQ ID Nos. 1-850), as well as full length cDNA, mRNA, and genes corresponding to these sequences, and to polypeptides and proteins encoded by these nucleic acids and genes and portions thereof.

Also included are nucleic acids that encode polypeptides and proteins encoded by the nucleic acids of SEQ ID Nos. 1-850. The various nucleic acids that can encode these polypeptides and proteins differ because of the degeneracy of the genetic code, in that most amino acids are encoded by more than one triplet codon. The identity of such codons is well known in this art, and this information can be used for the construction of the nucleic acids within the scope of the invention.

Nucleic acids encoding polypeptides and proteins that are variants of the polypeptides and proteins encoded by the nucleic acids and related cDNA and genes are also within the scope of the invention. The variants differ from wild-type protein in having one or more amino acid substitutions that either enhance, add, or diminish a biological activity of the wild-type protein. Once the amino acid change is selected, a nucleic acid encoding that variant is constructed according to the invention.

The following detailed description discloses how to obtain or make full-length cDNA and human genes corresponding to the nucleic acids, how to express these nucleic acids and genes, how to identify structural motifs of the genes, how to identify the function of a protein encoded by a gene corresponding to an nucleic acid, how to use nucleic acids as probes in mapping and in tissue profiling, how to use the corresponding polypeptides and proteins to raise antibodies, and how to use the nucleic acids, polypeptides, and proteins for therapeutic and diagnostic purposes.

The sequences investigated herein have been found to be differentially expressed in samples obtained from colon cancer cell lines and/or colon cancer tissue. However, it is also believed that these sequences may also have utility with other types of cancer.

Accordingly, certain aspects of the present invention relate to nucleic acids differentially expressed in tumor tissue, especially colon cancer cell lines, polypeptides encoded by such nucleic acids, and antibodies immunoreactive with these polypeptides, and preparations of such compositions. Moreover, the present invention provides diagnostic and therapeutic assays and reagents for detecting and treating disorders involving, for example, aberrant expression of the subject nucleic acids.

I. General

This invention relates in part to novel methods for identifying and/or classifying cancerous cells present in a human tumors, particularly in solid tumors, e.g., carcinomas and sarcomas, such as, for example, breast or colon cancers. The method uses genes that are differentially expressed in cancer cell lines and/or cancer tissue compared with related normal cells, such as normal colon cells, and thereby identifies or classifies tumor cells by the upregulation and/or downregulation of expression of particular genes, an event which is implicated in tumorigenesis.

Upregulation or increased expression of certain genes such as oncogenes, act to promote malignant growth. Downregulation or decreased expression of genes such as tumor suppressor genes promotes malignant growth. Thus, alteration in the expression of either type of gene is a potential diagnostic indicator for determining whether a subject is at risk of developing or has cancer, e.g., colon cancer.

Accordingly, in one aspect, the invention also provides biomarkers, such as nucleic acid markers, for human tumor cells, e.g., for colon cancer cells. The invention also provides proteins encoded by these nucleic acid markers.

The invention also features methods for identifying drugs useful for treatment of such cancer cells, and for treatment of a cancerous condition, such as colon cancer. Unlike prior methods, the invention provides a means for identifying cancer cells at an early stage of development, so that premalignant cells can be identified prior to their spreading throughout the human body. This allows early detection of potentially cancerous conditions, and treatment of those cancerous conditions prior to spread of the cancerous cells throughout the body, or prior to development of an irreversible cancerous condition.

II. Definitions

For convenience, the meaning of certain terms and phrases used in the specification, examples, and appended claims, are provided below.

The term "an aberrant expression", as applied to a nucleic acid of the present invention, refers to level of expression of that nucleic acid which differs from the level of expression of that nucleic acid in healthy tissue, or which differs from the activity of the polypeptide present in a healthy subject. An activity of a polypeptide can be aberrant because it is stronger than the activity of its native counterpart. Alternatively, an activity can be aberrant because it is weaker or absent relative to the activity of its native counterpart. An aberrant activity can also be a change in the activity; for example, an aberrant polypeptide can interact with a different target peptide. A cell can have an aberrant expression level of a gene due to overexpression or underexpression of that gene.

The term "agonist", as used herein, is meant to refer to an agent that mimics or upregulates (e.g., potentiates or supplements) the bioactivity of a protein. An agonist can be a wild-type protein or derivative thereof having at least one bioactivity of the wild-type protein. An agonist can also be a compound that upregulates expression of a gene or which increases at least one bioactivity of a protein. An agonist can also be a compound which increases the interaction of a polypeptide with another molecule, e.g., a target peptide or nucleic acid.

The term "allele", which is used interchangeably herein with "allelic variant", refers to alternative forms of a gene or portions thereof. Alleles occupy the same locus or position on homologous chromosomes. When a subject has two identical alleles of a gene, the subject is said to be homozygous for that gene or allele. When a subject has two different alleles of a gene, the subject is said to be heterozygous for the gene. Alleles of a specific gene can differ from each other in a single nucleotide, or several nucleotides, and can include substitutions, deletions, and/or insertions of nucleotides. An allele of a gene can also be a form of a gene containing mutations.

The term "allelic variant of a polymorphic region of a gene" refers to a region of a gene having one of several nucleotide sequences found in that region of the gene in other individuals.

"Antagonist" as used herein is meant to refer to an agent that downregulates (e.g., suppresses or inhibits) at least one bioactivity of a protein. An antagonist can be a compound which inhibits or decreases the interaction between a protein and another molecule, e.g., a target peptide or enzyme substrate. An antagonist can also be a compound that downregulates expression of a gene or which reduces the amount of expressed protein present.

The term "antibody" as used herein is intended to include whole antibodies, e.g., of any isotype (IgG, IgA, IgM, IgE, etc), and includes fragments thereof which are also specifically reactive with a vertebrate, e.g., mammalian, protein. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. Thus, the term includes segments of proteolytically-cleaved or recombinantly-prepared portions of an antibody molecule that are capable of selectively reacting with a certain protein. Nonlimiting examples of such proteolytic and/or recombinant fragments include Fab, F(ab')2, Fab' , Fv, and single chain antibodies (scFv) containing a V[L] and/or V[H] domain joined by a peptide linker. The scFv's may be covalently or non-covalently linked to form antibodies having two or more binding sites. The subject invention includes polyclonal, monoclonal, or other purified preparations of antibodies and recombinant antibodies.

The phenomenon of "apoptosis" is well known, and can be described as a programmed death of cells. As is known, apoptosis is contrasted with "necrosis", a phenomenon when cells die as a result of being killed by a toxic material, or other external effect. Apoptosis involves chromatic condensation, membrane blebbing, and fragmentation of DNA, all of which are generally visible upon microscopic examination.

A disease, disorder, or condition "associated with" or "characterized by" an aberrant expression of a nucleic acid refers to a disease, disorder, or condition in a subject which is caused by, contributed to by, or causative of an aberrant level of expression of a nucleic acid.

As used herein the term "bioactive fragment of a polypeptide" refers to a fragment of a full-length polypeptide, wherein the fragment specifically agonizes (mimics) or antagonizes (inhibits) the activity of a wild-type polypeptide. The

bioactive fragment preferably is a fragment capable of interacting with at least one other molecule, e.g., protein, small molecule, or DNA, which a full length protein can bind.

"Biological activity" or "bioactivity" or "activity" or "biological function", which are used interchangeably, herein mean an effector or antigenic function that is directly or indirectly performed by a polypeptide (whether in its native or denatured conformation), or by any subsequence thereof. Biological activities include binding to polypeptides, binding to other proteins or molecules, activity as a DNA binding protein, as a transcription regulator, ability to bind damaged DNA, etc. A bioactivity can be modulated by directly affecting the subject polypeptide. Alternatively, a bioactivity can be altered by modulating the level of the polypeptide, such as by modulating expression of the corresponding gene.

The term "biomarker" refers a biological molecule, e.g., a nucleic acid, peptide, hormone, etc., whose presence or concentration can be detected and correlated with a known condition, such as a disease state.

"Cells," "host cells", or "recombinant host cells" are terms used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A "chimeric polypeptide" or "fusion polypeptide" is a fusion of a first amino acid sequence encoding one of the subject polypeptides with a second amino acid sequence defining a domain (e.g., polypeptide portion) foreign to and not substantially homologous with any domain of the subject polypeptide. A chimeric polypeptide may present a foreign domain which is found (albeit in a different polypeptide) in an organism which also expresses the first polypeptide, or it may be an "interspecies," "intergenic," etc., fusion of polypeptide structures expressed by different kinds of organisms. In general, a fusion polypeptide can be represented by the general formula $(X)_n$-$(Y)_m$-$(Z)_n$, wherein Y represents a portion of the subject polypeptide, and X and Z are each independently absent or represent amino acid sequences which are not related to the native sequence found in an organism, or which are not found as a polypeptide

chain contiguous with the subject sequence, where m is an integer greater than or equal to one, and each occurrence of n is, independently, 0 or an integer greater than or equal to 1 (n and m are preferably no greater than 5 or 10).

A "delivery complex" shall mean a targeting means (e.g., a molecule that results in higher affinity binding of a nucleic acid, protein, polypeptide or peptide to a target cell surface and/or increased cellular or nuclear uptake by a target cell). Examples of targeting means include: sterols (e.g., cholesterol), lipids (e.g., a cationic lipid, virosome or liposome), viruses (e.g., adenovirus, adeno-associated virus, and retrovirus), or target cell-specific binding agents (e.g., ligands recognized by target cell specific receptors). Preferred complexes are sufficiently stable *in vivo* to prevent significant uncoupling prior to internalization by the target cell. However, the complex is cleavable under appropriate conditions within the cell so that the nucleic acid, protein, polypeptide or peptide is released in a functional form.

As is well known, genes or a particular polypeptide may exist in single or multiple copies within the genome of an individual. Such duplicate genes may be identical or may have certain modifications, including nucleotide substitutions, additions or deletions, which all still code for polypeptides having substantially the same activity. The term "DNA sequence encoding a polypeptide" may thus refer to one or more genes within a particular individual. Moreover, certain differences in nucleotide sequences may exist between individual organisms, which are called alleles. Such allelic differences may or may not result in differences in amino acid sequence of the encoded polypeptide yet still encode a polypeptide with the same biological activity.

The term "equivalent" is understood to include nucleotide sequences encoding functionally equivalent polypeptides. Equivalent nucleotide sequences will include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants; and will, therefore, include sequences that differ from the nucleotide sequence of the nucleic acids shown in SEQ ID NOs: 1-850 due to the degeneracy of the genetic code.

As used herein, the terms "gene", "recombinant gene", and "gene construct" refer to a nucleic acid of the present invention associated with an open reading frame, including both exon and (optionally) intron sequences.

A "recombinant gene" refers to nucleic acid encoding a polypeptide and comprising exon sequences, though it may optionally include intron sequences which are derived from, for example, a related or unrelated chromosomal gene. The term "intron" refers to a DNA sequence present in a given gene which is not translated into protein and is generally found between exons.

The term "growth" or "growth state" of a cell refers to the proliferative state of a cell as well as to its differentiative state. Accordingly, the term refers to the phase of the cell cycle in which the cell is, e.g., G0, G1, G2, prophase, metaphase, or telophase, as well as to its state of differentiation, e.g., undifferentiated, partially differentiated, or fully differentiated. Without wanting to be limited, differentiation of a cell is usually accompanied by a decrease in the proliferative rate of a cell.

"Homology" or "identity" or "similarity" refers to sequence similarity between two peptides or between two nucleic acid molecules, with identity being a more strict comparison. Homology and identity can each be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are identical at that position. A degree of homology or similarity or identity between nucleic acid sequences is a function of the number of identical or matching nucleotides at positions shared by the nucleic acid sequences. A degree of identity of amino acid sequences is a function of the number of identical amino acids at positions shared by the amino acid sequences. A degree of homology or similarity of amino acid sequences is a function of the number of amino acids, i.e., structurally related, at positions shared by the amino acid sequences. An "unrelated" or "non-homologous" sequence shares less than 40% identity, though preferably less than 25% identity, with one of the sequences of the present invention.

The term "percent identical" refers to sequence identity between two amino acid sequences or between two nucleotide sequences. Identity can each be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When an equivalent position in the compared sequences is occupied by the same base or amino acid, then the molecules are identical at that position; when the equivalent site occupied by the same or a similar amino acid residue (e.g., similar in steric and/or electronic nature), then the molecules can be referred to as

homologous (similar) at that position. Expression as a percentage of homology, similarity, or identity refers to a function of the number of identical or similar amino acids at positions shared by the compared sequences. Various alignment algorithms and/or programs may be used, including FASTA, BLAST, or ENTREZ. FASTA and BLAST are available as a part of the GCG sequence analysis package (University of Wisconsin, Madison, Wis.), and can be used with, e.g., default settings. ENTREZ is available through the National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Md. In one embodiment, the percent identity of two sequences can be determined by the GCG program with a gap weight of 1, e.g., each amino acid gap is weighted as if it were a single amino acid or nucleotide mismatch between the two sequences.

Other techniques for alignment are described in Methods in Enzymology, vol. 266: Computer Methods for Macromolecular Sequence Analysis (1996), ed. Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co., San Diego, California, USA. Preferably, an alignment program that permits gaps in the sequence is utilized to align the sequences. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See Meth. Mol. Biol. 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. An alternative search strategy uses MPSRCH software, which runs on a MASPAR computer. MPSRCH uses a Smith-Waterman algorithm to score sequences on a massively parallel computer. This approach improves ability to pick up distantly related matches, and is especially tolerant of small gaps and nucleotide sequence errors. Nucleic acid-encoded amino acid sequences can be used to search both protein and DNA databases.

Databases with individual sequences are described in Methods in Enzymology, ed. Doolittle, *supra*. Databases include Genbank, EMBL, and DNA Database of Japan (DDBJ).

Preferred nucleic acids have a sequence at least 70%, and more preferably 80% identical and more preferably 90% and even more preferably at least 95% identical to an nucleic acid sequence of a sequence shown in one of SEQ ID NOS: 1-850. Nucleic acids at least 90%, more preferably 95%, and most preferably at least about 98-99% identical with a nucleic sequence represented in one of SEQ ID NOS:

1-850 are of course also within the scope of the invention. In preferred embodiments, the nucleic acid is mammalian.

The term "interact" as used herein is meant to include detectable interactions (e.g., biochemical interactions) between molecules, such as interaction between protein-protein, protein-nucleic acid, nucleic acid-nucleic acid, and protein-small molecule or nucleic acid-small molecule in nature.

The term "isolated" as used herein with respect to nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs, or RNAs, respectively, that are present in the natural source of the macromolecule. The term isolated as used herein also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an "isolated nucleic acid" is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term "isolated" is also used herein to refer to polypeptides which are isolated from other cellular proteins and is meant to encompass both purified and recombinant polypeptides.

The terms "modulated" and "differentially regulated" as used herein refer to both upregulation (i.e., activation or stimulation (e.g., by agonizing or potentiating)) and downregulation (i.e., inhibition or suppression (e.g., by antagonizing, decreasing or inhibiting)).

The term "mutated gene" refers to an allelic form of a gene, which is capable of altering the phenotype of a subject having the mutated gene relative to a subject which does not have the mutated gene. If a subject must be homozygous for this mutation to have an altered phenotype, the mutation is said to be recessive. If one copy of the mutated gene is sufficient to alter the genotype of the subject, the mutation is said to be dominant. If a subject has one copy of the mutated gene and has a phenotype that is intermediate between that of a homozygous and that of a heterozygous subject (for that gene), the mutation is said to be co-dominant.

The designation "N", where it appears in the accompanying Sequence Listing, indicates that the identity of the corresponding nucleotide is unknown. "N" should therefore not necessarily be interpreted as permitting substitution with any nucleotide,

e.g., A, T, C, or G, but rather as holding the place of a nucleotide whose identity has not been conclusively determined.

The "non-human animals" of the invention include mammalians such as rodents, non-human primates, sheep, dog, cow, chickens, amphibians, reptiles, etc. Preferred non-human animals are selected from the rodent family including rat and mouse, most preferably mouse, though transgenic amphibians, such as members of the *Xenopus* genus, and transgenic chickens can also provide important tools for understanding and identifying agents which can affect, for example, embryogenesis and tissue formation. The term "chimeric animal" is used herein to refer to animals in which the recombinant gene is found, or in which the recombinant gene is expressed in some but not all cells of the animal. The term "tissue-specific chimeric animal" indicates that one of the recombinant genes is present and/or expressed or disrupted in some tissues but not others.

As used herein, the term "nucleic acid" refers to polynucleotides such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single (sense or antisense) and double-stranded polynucleotides. ESTs, chromosomes, cDNAs, mRNAs, and rRNAs are representative examples of molecules that may be referred to as nucleic acids.

The term "nucleotide sequence complementary to the nucleotide sequence of SEQ ID NO. x" refers to the nucleotide sequence of the complementary strand of a nucleic acid strand having SEQ ID NO. x. The term "complementary strand" is used herein interchangeably with the term "complement". The complement of a nucleic acid strand can be the complement of a coding strand or the complement of a non-coding strand.

The term "polymorphism" refers to the coexistence of more than one form of a gene or portion (e.g., allelic variant) thereof. A portion of a gene of which there are at least two different forms, i.e., two different nucleotide sequences, is referred to as a "polymorphic region of a gene". A polymorphic region can be a single nucleotide, the identity of which differs in different alleles. A polymorphic region can also be several nucleotides long.

A "polymorphic gene" refers to a gene having at least one polymorphic region.

As used herein, the term "promoter" means a DNA sequence that regulates expression of a selected DNA sequence operably linked to the promoter, and which effects expression of the selected DNA sequence in cells. The term encompasses "tissue specific" promoters, i.e., promoters which effect expression of the selected DNA sequence only in specific cells (e.g., cells of a specific tissue). The term also covers so-called "leaky" promoters, which regulate expression of a selected DNA primarily in one tissue, but cause expression in other tissues as well. The term also encompasses non-tissue specific promoters and promoters that constitutively express or that are inducible (i.e., expression levels can be controlled).

The terms "protein", "polypeptide", and "peptide" are used interchangeably herein when referring to a gene product.

The term "recombinant protein" refers to a polypeptide of the present invention which is produced by recombinant DNA techniques, wherein generally, DNA encoding a polypeptide is inserted into a suitable expression vector which is in turn used to transform a host cell to produce the heterologous protein. Moreover, the phrase "derived from", with respect to a recombinant gene, is meant to include within the meaning of "recombinant protein" those proteins having an amino acid sequence of a native polypeptide, or an amino acid sequence similar thereto which is generated by mutations including substitutions and deletions (including truncation) of a naturally occurring form of the polypeptide.

"Small molecule" as used herein, is meant to refer to a composition, which has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic (carbon-containing) or inorganic molecules. Many pharmaceutical companies have extensive libraries of chemical and/or biological mixtures, often fungal, bacterial, or algal extracts, which can be screened with any of the assays of the invention to identify compounds that modulate a bioactivity.

As used herein, the term "specifically hybridizes" or "specifically detects" refers to the ability of a nucleic acid molecule of the invention to hybridize to at least a portion of, for example approximately 6, 12, 15, 20, 30, 50, 100, 150, 200, 300, 350,

400, 500, 750 or 1000 contiguous nucleotides of a nucleic acid designated in any one of SEQ ID Nos: 1-850, or a sequence complementary thereto, or naturally occurring mutants thereof, such that it has less than 15%, preferably less than 10%, and more preferably less than 5% background hybridization to a cellular nucleic acid (e.g., mRNA or genomic DNA) encoding a different protein. In preferred embodiments, the oligonucleotide probe detects only a specific nucleic acid, e.g., it does not substantially hybridize to similar or related nucleic acids, or complements thereof.

"Transcriptional regulatory sequence" is a generic term used throughout the specification to refer to DNA sequences, such as initiation signals, enhancers, and promoters, which induce or control transcription of protein coding sequences with which they are operably linked. In preferred embodiments, transcription of one of the genes is under the control of a promoter sequence (or other transcriptional regulatory sequence) which controls the expression of the recombinant gene in a cell-type in which expression is intended. It will also be understood that the recombinant gene can be under the control of transcriptional regulatory sequences which are the same or which are different from those sequences which control transcription of the naturally-occurring forms of the polypeptide.

As used herein, the term "transfection" means the introduction of a nucleic acid, e.g., via an expression vector, into a recipient cell by nucleic acid-mediated gene transfer. "Transformation", as used herein, refers to a process in which a cell's genotype is changed as a result of the cellular uptake of exogenous DNA or RNA, and, for example, the transformed cell expresses a recombinant form of a polypeptide or, in the case of anti-sense expression from the transferred gene, the expression of the target gene is disrupted.

As used herein, the term "transgene" means a nucleic acid sequence (or an antisense transcript thereto) which has been introduced into a cell. A transgene could be partly or entirely heterologous, i.e., foreign, to the transgenic animal or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the animal's genome in such a way as to alter the genome of the cell into which it is inserted (e.g., it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout). A transgene can also be present in

a cell in the form of an episome. A transgene can include one or more transcriptional regulatory sequences and any other nucleic acid, such as introns, that may be necessary for optimal expression of a selected nucleic acid.

A "transgenic animal" refers to any animal, preferably a non-human mammal, bird or an amphibian, in which one or more of the cells of the animal contain heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or *in vitro* fertilization, but rather is directed to the introduction of a recombinant DNA molecule. This molecule may be integrated within a chromosome, or it may be extra-chromosomally replicating DNA. In the typical transgenic animals described herein, the transgene causes cells to express a recombinant form of one of the subject polypeptide, e.g. either agonistic or antagonistic forms. However, transgenic animals in which the recombinant gene is silent are also contemplated, as for example, the FLP or CRE recombinase dependent constructs described below. Moreover, "transgenic animal" also includes those recombinant animals in which gene disruption of one or more genes is caused by human intervention, including both recombination and antisense techniques.

The term "treating" as used herein is intended to encompass curing as well as ameliorating at least one symptom of the condition or disease.

The term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of preferred vector is an episome, i.e., a nucleic acid capable of extra-chromosomal replication. Preferred vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of "plasmids" which refer generally to circular double stranded DNA loops which, in their vector form are not bound to the chromosome. In the present specification, "plasmid" and "vector" are used interchangeably as the plasmid is the

most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which become known in the art subsequently hereto.

The term "wild-type allele" refers to an allele of a gene which, when present in two copies in a subject results in a wild-type phenotype. There can be several different wild-type alleles of a specific gene, since certain nucleotide changes in a gene may not affect the phenotype of a subject having two copies of the gene with the nucleotide changes.

III. Nucleic Acids of the Present Invention

As described below, one aspect of the invention pertains to isolated nucleic acids, variants, and/or equivalents of such nucleic acids.

Nucleic acids of the present invention have been identified as differentially expressed in tumor cells, e.g., colon cancer-derived cell lines (relative to the expression levels in normal tissue, e.g., normal colon tissue and/or normal non-colon tissue), such as SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto. In certain embodiments, the subject nucleic acids are differentially expressed by at least a factor of two, preferably at least a factor of five, even more preferably at least a factor of twenty, still more preferably at least a factor of fifty. Preferred nucleic acids include sequences identified as differentially expressed both in colon cancer cell tissue and colon cancer cell lines. In preferred embodiments, nucleic acids of the present invention are upregulated in tumor cells, especially colon cancer tissue and/or colon cancer-derived cell lines. In another embodiment, nucleic acids of the present invention are downregulated in tumor cells, especially colon cancer tissue and/or colon cancer-derived cell lines.

Table 1 indicates those sequences which are over- or underexpressed in a colon cancer-derived cell line relative to normal tissue, and further designates those sequences which are also differentially regulated in colon cancer tissue. The designation O indicates that the corresponding sequence was overexpressed, M indicates possible overexpression, N indicates no differential expression, and U indicates underexpression.

Genes which are upregulated, such as oncogenes, or downregulated, such as tumor suppressors, in aberrantly proliferating cells may be targets for diagnostic or therapeutic techniques. For example, upregulation of the *cdc2* gene induces mitosis. Overexpression of the *myt1* gene, a mitotic deactivator, negatively regulates the activity of *cdc2*. Aberrant proliferation may thus be induced either by upregulating *cdc2* or by downregulating *myt1*. Similarly, downregulation of tumor suppressors such as *p53* and *Rb* have been implicated in tumorigenesis.

Particularly preferred polypeptides are those that are encoded by nucleic acid sequences at least about 70%, 75%, 80%, 90%, 95%, 97%, or 98% similar to a nucleic acid sequence of SEQ ID Nos. 1-850. Preferably, the nucleic acid includes all or a portion (e.g., at least about 12, at least about 15, at least about 25, or at least about 40 nucleotides) of the nucleotide sequence corresponding to the nucleic acid of SEQ ID Nos. 1-383, preferably SEQ ID Nos. 1-127, or a sequence complementary thereto.

Still other preferred nucleic acids of the present invention encode a polypeptide comprising at least a portion of a polypeptide encoded by one of SEQ ID Nos. 1-850. For example, preferred nucleic acid molecules for use as probes/primers or antisense molecules (i.e., noncoding nucleic acid molecules) can comprise at least about 12, 20, 30, 50, 60, 70, 80, 90, or 100 base pairs in length up to the length of the complete gene. Coding nucleic acid molecules can comprise, for example, from about 50, 60, 70, 80, 90, or 100 base pairs up to the length of the complete gene.

Another aspect of the invention provides a nucleic acid which hybridizes under low, medium, or high stringency conditions to a nucleic acid sequence represented by one of SEQ ID Nos. 1-383, preferably SEQ ID Nos. 1-127, or a sequence complementary thereto. Appropriate stringency conditions which promote DNA hybridization, for example, 6.0 x sodium chloride/sodium citrate (SSC) at about 45 °C, followed by a wash of 2.0 x SSC at 50 °C, are known to those skilled in the art or can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-12.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50 °C to a high stringency of about 0.2 x SSC at 50 °C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22 °C, to high stringency conditions at about 65 °C. Both temperature and salt may be varied, or

temperature or salt concentration may be held constant while the other variable is changed. In a preferred embodiment, a nucleic acid of the present invention will bind to one of SEQ ID Nos. 1-383, preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, under moderately stringent conditions, for example at about 2.0 x SSC and about 40 °C. In a particularly preferred embodiment, a nucleic acid of the present invention will bind to one of SEQ ID Nos. 1-383, preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, under high stringency conditions.

In one embodiment, the invention provides nucleic acids which hybridize under low stringency conditions of 6 x SSC at room temperature followed by a wash at 2 x SSC at room temperature.

In another embodiment, the invention provides nucleic acids which hybridize under high stringency conditions of 2 x SSC at 65 °C followed by a wash at 0.2 x SSC at 65 °C.

Nucleic acids having a sequence that differs from the nucleotide sequences shown in one of SEQ ID Nos. 1-383, preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, due to degeneracy in the genetic code, are also within the scope of the invention. Such nucleic acids encode functionally equivalent peptides (i.e., a peptide having equivalent or similar biological activity) but differ in sequence from the sequence shown in the sequence listing due to degeneracy in the genetic code. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC each encode histidine) may result in "silent" mutations which do not affect the amino acid sequence of a polypeptide. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the subject polypeptides will exist among mammals. One skilled in the art will appreciate that these variations in one or more nucleotides (e.g., up to about 3-5% of the nucleotides) of the nucleic acids encoding polypeptides having an activity of a polypeptide may exist among individuals of a given species due to natural allelic variation.

Also within the scope of the invention are nucleic acids encoding splicing variants of proteins encoded by a nucleic acid of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence

complementary thereto, or natural homologs of such proteins. Such homologs can be cloned by hybridization or PCR, as further described herein.

The polynucleotide sequence may also encode for a leader sequence, e.g., the natural leader sequence or a heterologous leader sequence, for a subject polypeptide. For example, the desired DNA sequence may be fused in the same reading frame to a DNA sequence which aids in expression and secretion of the polypeptide from the host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of the polypeptide from the cell. The protein having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the mature form of the protein.

The polynucleotide of the present invention may also be fused in frame to a marker sequence, also referred to herein as "Tag sequence" encoding a "Tag peptide", which allows for marking and/or purification of the polypeptide of the present invention. In a preferred embodiment, the marker sequence is a hexahistidine tag, e.g., supplied by a PQE-9 vector. Numerous other Tag peptides are available commercially. Other frequently used Tags include myc-epitopes (e.g., see Ellison et al. (1991) *J Biol Chem 266*:21150-21157) which includes a 10-residue sequence from c-myc, the pFLAG system (International Biotechnologies, Inc.), the pEZZ-protein A system (Pharmacia, NJ), and a 16 amino acid portion of the *Haemophilus influenza* hemagglutinin protein. Furthermore, any polypeptide can be used as a Tag so long as a reagent, e.g., an antibody interacting specifically with the Tag polypeptide is available or can be prepared or identified.

As indicated by the examples set out below, nucleic acids can be obtained from mRNA present in any of a number of eukaryotic cells, e.g., and are preferably obtained from metazoan cells, more preferably from vertebrate cells, and even more preferably from mammalian cells. It should also be possible to obtain nucleic acids of the present invention from genomic DNA from both adults and embryos. For example, a gene can be cloned from either a cDNA or a genomic library in accordance with protocols generally known to persons skilled in the art. cDNA can be obtained by isolating total mRNA from a cell, e.g., a vertebrate cell, a mammalian cell, or a human cell, including embryonic cells. Double stranded cDNAs can then be prepared from the total mRNA, and subsequently inserted into a suitable plasmid or bacteriophage

vector using any one of a number of known techniques. The gene can also be cloned using established polymerase chain reaction techniques in accordance with the nucleotide sequence information provided by the invention.

In certain embodiments, a nucleic acid, probe, vector, or other construct of the present invention includes at least about five, at least about ten, or at least about twenty nucleic acids from a region designated as novel in Table 2. In certain other embodiments, a nucleic acid of the present invention includes at least about five, at least about ten, or at least about twenty nucleic acids which are not included in the clones whose accession numbers are listed in Table 2.

The invention includes within its scope a polynucleotide having the nucleotide sequence of nucleic acid obtained from this biological material, wherein the nucleic acid hybridizes under stringent conditions (at least about 4 x SSC at 65°C, or at least about 4 x SSC at 42°C; see, for example, U.S. Patent No. 5,707,829, incorporated herein by reference) with at least 15 contiguous nucleotides of at least one of SEQ ID Nos. 1-850. By this is intended that when at least 15 contiguous nucleotides of one of SEQ ID Nos. 1-850 is used as a probe, the probe will preferentially hybridize with a gene or mRNA (of the biological material) comprising the complementary sequence, allowing the identification and retrieval of the nucleic acids of the biological material that uniquely hybridize to the selected probe. Probes from more than one of SEQ ID Nos. 1-850 will hybridize with the same gene or mRNA if the cDNA from which they were derived corresponds to one mRNA. Probes of more than 15 nucleotides can be used, but 15 nucleotides represents enough sequence for unique identification.

Because the present nucleic acids represent partial mRNA transcripts, two or more nucleic acids of the invention may represent different regions of the same mRNA transcript and the same gene. Thus, if two or more of SEQ ID Nos. 1-850 are identified as belonging to the same clone, then either sequence can be used to obtain the full-length mRNA or gene.

Nucleic acid-related polynucleotides can also be isolated from cDNA libraries. These libraries are preferably prepared from mRNA of human colon cells, more preferably, human colon cancer cells, even more preferably, from a human colon adenocarcinoma cell line, SW480. Alignment of SEQ ID Nos. 1-850, as described

above, can indicated that a cell line or tissue source of a related protein or polynucleotide can also be used as a source of the nucleic acid-related cDNA.

Techniques for producing and probing nucleic acid sequence libraries are described, for example, in Sambrook *et al.*, "Molecular Cloning: A Laboratory Manual" (New York, Cold Spring Harbor Laboratory, 1989). The cDNA can be prepared by using primers based on a sequence from SEQ ID Nos. 1-850. In one embodiment, the cDNA library can be made from only poly-adenylated mRNA. Thus, poly-T primers can be used to prepare cDNA from the mRNA. Alignment of SEQ ID Nos. 1-850 can result in identification of a related polypeptide or polynucleotide. Some of the polynucleotides disclosed herein contains repetitive regions that were subject to masking during the search procedures. The information about the repetitive regions is discussed below.

Constructs of polynucleotides having sequences of SEQ ID Nos. 1-850 can be generated synthetically. Alternatively, single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides is described by Stemmer et al., *Gene (Amsterdam)* (1995) *164(1)*:49-53. In this method, assembly PCR (the synthesis of long DNA sequences from large numbers of oligodeoxyribonucleotides (oligos)) is described. The method is derived from DNA shuffling (Stemmer, *Nature* (1994) *370*:389-391), and does not rely on DNA ligase, but instead relies on DNA polymerase to build increasingly longer DNA fragments during the assembly process. For example, a 1.1-kb fragment containing the TEM-1 beta-lactamase-encoding gene (bla) can be assembled in a single reaction from a total of 56 oligos, each 40 nucleotides (nt) in length. The synthetic gene can be PCR amplified and cloned in a vector containing the tetracycline-resistance gene (Tc-R) as the sole selectable marker. Without relying on ampicillin (Ap) selection, 76% of the Tc-R colonies were Ap-R, making this approach a general method for the rapid and cost-effective synthesis of any gene.

IV. Identification of Functional and Structural Motifs of Novel Genes Using Art-Recognized Methods

Translations of the nucleotide sequence of the nucleic acids, cDNAs, or full genes can be aligned with individual known sequences. Similarity with individual

sequences can be used to determine the activity of the polypeptides encoded by the polynucleotides of the invention. For example, sequences that show similarity with a chemokine sequence may exhibit chemokine activities. Also, sequences exhibiting similarity with more than one individual sequence may exhibit activities that are characteristic of either or both individual sequences.

The full length sequences and fragments of the polynucleotide sequences of the nearest neighbors can be used as probes and primers to identify and isolate the full length sequence of the nucleic acid. The nearest neighbors can indicate a tissue or cell type to be used to construct a library for the full-length sequences of the nucleic acid.

Typically, the nucleic acids are translated in all six frames to determine the best alignment with the individual sequences. The sequences disclosed herein in the Sequence Listing are in a 5' to 3' orientation and translation in three frames can be sufficient (with a few specific exceptions as described in the Examples). These amino acid sequences are referred to, generally, as query sequences, which will be aligned with the individual sequences.

Nucleic acid sequences can be compared with known genes by any of the methods disclosed above. Results of individual and query sequence alignments can be divided into three categories: high similarity, weak similarity, and no similarity. Individual alignment results ranging from high similarity to weak similarity provide a basis for determining polypeptide activity and/or structure.

Parameters for categorizing individual results include: percentage of the alignment region length where the strongest alignment is found, percent sequence identity, and p value.

The percentage of the alignment region length is calculated by counting the number of residues of the individual sequence found in the region of strongest alignment. This number is divided by the total residue length of the query sequence to find a percentage. An example is shown below:

```
Query sequence:          ASNPERTMIPVTRVGLIRYM
                             |   ||| |||| |||
Individual sequence:     YMMTEYLAIPV.RVGLPRYM
                         1   5   10    15
```

The region of alignment begins at amino acid 9 and ends at amino acid 19. The total length of the query sequence is 20 amino acids. The percent of the alignment region length is 11/20 or 55%.

Percent sequence identity is calculated by counting the number of amino acid matches between the query and individual sequence and dividing total number of matches by the number of residues of the individual sequence found in the region of strongest alignment. For the example above, the percent identity would be 10 matches divided by 11 amino acids, or approximately 90.9%.

P value is the probability that the alignment was produced by chance. For a single alignment, the p value can be calculated according to Karlin *et al.*, Proc. Natl. Acad. Sci. 87: 2264 (1990) and Karlin *et al.*, Proc. Natl. Acad. Sci. 90: (1993). The p value of multiple alignments using the same query sequence can be calculated using an heuristic approach described in Altschul *et al.*, Nat. Genet. 6: 119 (1994). Alignment programs such as BLAST program can calculate the p value.

The boundaries of the region where the sequences align can be determined according to Doolittle, Methods in Enzymology, *supra*; BLAST or FASTA programs; or by determining the area where the sequence identity is highest.

Another factor to consider for determining identity or similarity is the location of the similarity or identity. Strong local alignment can indicate similarity even if the length of alignment is short. Sequence identity scattered throughout the length of the query sequence also can indicate a similarity between the query and profile sequences.

High Similarity**Error! Bookmark not defined.**

For the alignment results to be considered high similarity, the percent of the alignment region length, typically, is at least about 55% of total length query sequence; more typically, at least about 58%; even more typically; at least about 60% of the total residue length of the query sequence. Usually, percent length of the alignment region can be as much as about 62%; more usually, as much as about 64%; even more usually, as much as about 66%.

Further, for high similarity, the region of alignment, typically, exhibits at least about 75% of sequence identity; more typically, at least about 78%; even more typically; at least about 80% sequence identity. Usually, percent sequence identity

can be as much as about 82%; more usually, as much as about 84%; even more usually, as much as about 86%.

The p value is used in conjunction with these methods. If high similarity is found, the query sequence is considered to have high similarity with a profile sequence when the p value is less than or equal to about 10^{-2}; more usually; less than or equal to about 10^{-3}; even more usually; less than or equal to about 10^{-4}. More typically, the p value is no more than about 10^{-5}; more typically; no more than or equal to about 10^{-10}; even more typically; no more than or equal to about 10^{-15} for the query sequence to be considered high similarity.

Weak Similarity

For the alignment results to be considered weak similarity, there is no minimum percent length of the alignment region nor minimum length of alignment. A better showing of weak similarity is considered when the region of alignment is, typically, at least about 15 amino acid residues in length; more typically, at least about 20; even more typically; at least about 25 amino acid residues in length. Usually, length of the alignment region can be as much as about 30 amino acid residues; more usually, as much as about 40; even more usually, as much as about 60 amino acid residues.

Further, for weak similarity, the region of alignment, typically, exhibits at least about 35% of sequence identity; more typically, at least about 40%; even more typically; at least about 45% sequence identity. Usually, percent sequence identity can be as much as about 50%; more usually, as much as about 55%; even more usually, as much as about 60%.

If low similarity is found, the query sequence is considered to have weak similarity with a profile sequence when the p value is usually less than or equal to about 10^{-2}; more usually; less than or equal to about 10^{-3}; even more usually; less than or equal to about 10^{-4}. More typically, the p value is no more than about 10^{-5}; more usually; no more than or equal to about 10^{-10}; even more usually; no more than or equal to about 10^{-15} for the query sequence to be considered weak similarity.

Similarity Determined by Sequence Identity Alone**Error! Bookmark not defined.**

Sequence identity alone can be used to determine similarity of a query sequence to an individual sequence and can indicate the activity of the sequence. Such an alignment, preferably, permits gaps to align sequences. Typically, the query sequence is related to the profile sequence if the sequence identity over the entire query sequence is at least about 15%; more typically, at least about 20%; even more typically, at least about 25%; even more typically, at least about 50%. Sequence identity alone as a measure of similarity is most useful when the query sequence is usually, at least 80 residues in length; more usually, 90 residues; even more usually, at least 95 amino acid residues in length. More typically, similarity can be concluded based on sequence identity alone when the query sequence is preferably 100 residues in length; more preferably, 120 residues in length; even more preferably, 150 amino acid residues in length.

Determining Activity from Alignments with Profile and Multiple Aligned Sequences

Translations of the nucleic acids can be aligned with amino acid profiles that define either protein families or common motifs. Also, translations of the nucleic acids can be aligned to multiple sequence alignments (MSA) comprising the polypeptide sequences of members of protein families or motifs. Similarity or identity with profile sequences or MSAs can be used to determine the activity of the polypeptides encoded by nucleic acids or corresponding cDNA or genes. For example, sequences that show an identity or similarity with a chemokine profile or MSA can exhibit chemokine activities.

Profiles can designed manually by (1) creating a MSA, which is an alignment of the amino acid sequence of members that belong to the family and (2) constructing a statistical representation of the alignment. Such methods are described, for example, in Birney *et al.*, Nucl. Acid Res. 24(14): 2730-2739 (1996).

MSAs of some protein families and motifs are publicly available. For example, these include MSAs of 547 different families and motifs. These MSAs are described also in Sonnhammer *et al.*, Proteins 28: 405-420 (1997). Other sources are also available in the world wide web. A brief description of these MSAs is reported in Pascarella *et al.*, Prot. Eng. 9(3): 249-251 (1996).

Techniques for building profiles from MSAs are described in Sonnhammer *et al.*, *supra*; Birney *et al.*, *supra*; and Methods in Enzymology, vol. 266: "Computer Methods for Macromolecular Sequence Analysis," 1996, ed. Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co., San Diego, California, USA.

Similarity between a query sequence and a protein family or motif can be determined by (a) comparing the query sequence against the profile and/or (b) aligning the query sequence with the members of the family or motif.

Typically, a program such as Searchwise can be used to compare the query sequence to the statistical representation of the multiple alignment, also known as a profile. The program is described in Birney *et al.*, *supra*. Other techniques to compare the sequence and profile are described in Sonnhammer *et al.*, *supra* and Doolittle, *supra*.

Next, methods described by Feng *et al.*, J. Mol. Evol. 25: 351-360 (1987) and Higgins *et al.*, CABIOS 5: 151-153 (1989) can be used align the query sequence with the members of a family or motif, also known as a MSA. Computer programs, such as PILEUP, can be used. See Feng *et al.*, *infra*.

The following factors are used to determine if a similarity between a query sequence and a profile or MSA exists: (1) number of conserved residues found in the query sequence, (2) percentage of conserved residues found in the query sequence, (3) number of frameshifts, and (4) spacing between conserved residues.

Some alignment programs that both translate and align sequences can make any number of frameshifts when translating the nucleotide sequence to produce the best alignment. The fewer frameshifts needed to produce an alignment, the stronger the similarity or identity between the query and profile or MSAs. For example, a weak similarity resulting from no frameshifts can be a better indication of activity or structure of a query sequence, than a strong similarity resulting from two frameshifts. Preferably, three or fewer frameshifts are found in an alignment; more preferably two or fewer frameshifts; even more preferably, one or fewer frameshifts; even more preferably, no frameshifts are found in an alignment of query and profile or MSAs.

Conserved residues are those amino acids that are found at a particular position in all or some of the family or motif members. For example, most known chemokines contain four conserved cysteines. Alternatively, a position is considered

conserved if only a certain class of amino acids is found in a particular position in all or some of the family members. For example, the N-terminal position may contain a positively charged amino acid, such as lysine, arginine, or histidine.

Typically, a residue of a polypeptide is conserved when a class of amino acids or a single amino acid is found at a particular position in at least about 40% of all class members; more typically, at least about 50%; even more typically, at least about 60% of the members. Usually, a residue is conserved when a class or single amino acid is found in at least about 70% of the members of a family or motif; more usually, at least about 80%; even more usually, at least about 90%; even more usually, at least about 95%.

A residue is considered conserved when three unrelated amino acids are found at a particular position in the some or all of the members; more usually, two unrelated amino acids. These residues are conserved when the unrelated amino acids are found at particular positions in at least about 40% of all class member; more typically, at least about 50%; even more typically, at least about 60% of the members. Usually, a residue is conserved when a class or single amino acid is found in at least about 70% of the members of a family or motif; more usually, at least about 80%; even more usually, at least about 90%; even more usually, at least about 95%.

A query sequence has similarity to a profile or MSA when the query sequence comprises at least about 25% of the conserved residues of the profile or MSA; more usually, at least about 30%; even more usually; at least about 40%. Typically, the query sequence has a stronger similarity to a profile sequence or MSA when the query sequence comprises at least about 45% of the conserved residues of the profile or MSA; more typically, at least about 50%; even more typically; at least about 55%.

V. Probes and Primers

The nucleotide sequences determined from the cloning of genes from tumor cells, especially colon cancer cell lines and tissues will further allow for the generation of probes and primers designed for identifying and/or cloning homologs in other cell types, e.g., from other tissues, as well as homologs from other mammalian organisms. Nucleotide sequences useful as probes/primers may include all or a portion of the sequences listed in SEQ ID Nos. 1-850 or sequences complementary

thereto or sequences which hybridize under stringent conditions to all or a portion of SEQ ID Nos. 1-850. For instance, the present invention also provides a probe/primer comprising a substantially purified oligonucleotide, which oligonucleotide comprising a nucleotide sequence that hybridizes under stringent conditions to at least approximately 12, preferably 25, more preferably 40, 50, or 75 consecutive nucleotides up to the full length of the sense or anti-sense sequence selected from the group consisting of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, or naturally occurring mutants thereof. For instance, primers based on a nucleic acid represented in SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, can be used in PCR reactions to clone homologs of that sequence.

In yet another embodiment, the invention provides probes/primers comprising a nucleotide sequence that hybridizes under moderately stringent conditions to at least approximately 12, 16, 25, 40, 50 or 75 consecutive nucleotides up to the full length of the sense or antisense sequence selected from the group consisting of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or naturally occurring mutants thereof.

In particular, these probes are useful because they provide a method for detecting mutations in wild-type genes of the present invention. Nucleic acid probes which are complementary to a wild-type gene of the present invention and can form mismatches with mutant genes are provided, allowing for detection by enzymatic or chemical cleavage or by shifts in electrophoretic mobility.

Likewise, probes based on the subject sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins, for use, for example, in prognostic or diagnostic assays. In preferred embodiments, the probe further comprises a label group attached thereto and able to be detected, e.g., the label group is selected from radioisotopes, fluorescent compounds, chemiluminescent compounds, enzymes, and enzyme co-factors.

Full-length cDNA molecules comprising the disclosed nucleic acids are obtained as follows. A subject nucleic acid or a portion thereof comprising at least about 12, 15, 18, or 20 nucleotides up to the full length of a sequence represented in

SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, may be used as a hybridization probe to detect hybridizing members of a cDNA library using probe design methods, cloning methods, and clone selection techniques as described in U.S. Patent No. 5,654,173, "Secreted Proteins and Polynucleotides Encoding Them," incorporated herein by reference. Libraries of cDNA may be made from selected tissues, such as normal or tumor tissue, or from tissues of a mammal treated with, for example, a pharmaceutical agent. Preferably, the tissue is the same as that used to generate the nucleic acids, as both the nucleic acid and the cDNA represent expressed genes. Most preferably, the cDNA library is made from the biological material described herein in the Examples. Alternatively, many cDNA libraries are available commercially. (Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual, 2nd Ed.* (Cold Spring Harbor Press, Cold Spring Harbor, NY 1989). The choice of cell type for library construction may be made after the identity of the protein encoded by the nucleic acid-related gene is known. This will indicate which tissue and cell types are likely to express the related gene, thereby containing the mRNA for generating the cDNA.

Members of the library that are larger than the nucleic acid, and preferably that contain the whole sequence of the native message, may be obtained. To confirm that the entire cDNA has been obtained, RNA protection experiments may be performed as follows. Hybridization of a full-length cDNA to an mRNA may protect the RNA from RNase degradation. If the cDNA is not full length, then the portions of the mRNA that are not hybridized may be subject to RNase degradation. This may be assayed, as is known in the art, by changes in electrophoretic mobility on polyacrylamide gels, or by detection of released monoribonucleotides. Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual, 2nd Ed.* (Cold Spring Harbor Press, Cold Spring Harbor, NY 1989). In order to obtain additional sequences 5' to the end of a partial cDNA, 5' RACE (PCR Protocols: A Guide to Methods and Applications (Academic Press, Inc. 1990)) may be performed.

Genomic DNA may be isolated using nucleic acids in a manner similar to the isolation of full-length cDNAs. Briefly, the nucleic acids, or portions thereof, may be used as probes to libraries of genomic DNA. Preferably, the library is obtained from the cell type that was used to generate the nucleic acids. Most preferably, the genomic

DNA is obtained from the biological material described herein in the Example. Such libraries may be in vectors suitable for carrying large segments of a genome, such as P1 or YAC, as described in detail in Sambrook *et al.*, 9.4-9.30. In addition, genomic sequences can be isolated from human BAC libraries, which are commercially available from Research Genetics, Inc., Huntville, Alabama, USA, for example. In order to obtain additional 5' or 3' sequences, chromosome walking may be performed, as described in Sambrook *et al.*, such that adjacent and overlapping fragments of genomic DNA are isolated. These may be mapped and pieced together, as is known in the art, using restriction digestion enzymes and DNA ligase.

Using the nucleic acids of the invention, corresponding full length genes can be isolated using both classical and PCR methods to construct and probe cDNA libraries. Using either method, Northern blots, preferably, may be performed on a number of cell types to determine which cell lines express the gene of interest at the highest rate.

Classical methods of constructing cDNA libraries are taught in Sambrook et al., supra. With these methods, cDNA can be produced from mRNA and inserted into viral or expression vectors. Typically, libraries of mRNA comprising poly(A) tails can be produced with poly(T) primers. Similarly, cDNA libraries can be produced using the instant sequences as primers.

PCR methods may be used to amplify the members of a cDNA library that comprise the desired insert. In this case, the desired insert may contain sequence from the full length cDNA that corresponds to the instant nucleic acids. Such PCR methods include gene trapping and RACE methods.

Gene trapping may entail inserting a member of a cDNA library into a vector. The vector then may be denatured to produce single stranded molecules. Next, a substrate-bound probe, such a biotinylated oligo, may be used to trap cDNA inserts of interest. Biotinylated probes can be linked to an avidin-bound solid substrate. PCR methods can be used to amplify the trapped cDNA. To trap sequences corresponding to the full length genes, the labeled probe sequence may be based on the nucleic acids of the invention, e.g., SEQ ID Nos. 1-383, preferably SEQ ID Nos. 1-127, or a sequence complementary thereto. Random primers or primers specific to the library vector can be used to amplify the trapped cDNA. Such gene trapping techniques are

described in Gruber *et al.*, PCT WO 95/04745 and Gruber *et al.*, U.S. Pat. No. 5,500,356. Kits are commercially available to perform gene trapping experiments from, for example, Life Technologies, Gaithersburg, Maryland, USA.

"Rapid amplification of cDNA ends," or RACE, is a PCR method of amplifying cDNAs from a number of different RNAs. The cDNAs may be ligated to an oligonucleotide linker and amplified by PCR using two primers. One primer may be based on sequence from the instant nucleic acids, for which full length sequence is desired, and a second primer may comprise a sequence that hybridizes to the oligonucleotide linker to amplify the cDNA. A description of this method is reported in PCT Pub. No. WO 97/19110.

In preferred embodiments of RACE, a common primer may be designed to anneal to an arbitrary adaptor sequence ligated to cDNA ends (Apte and Siebert, Biotechniques 15:890-893, 1993; Edwards et al., Nuc. Acids Res. 19:5227-5232, 1991). When a single gene-specific RACE primer is paired with the common primer, preferential amplification of sequences between the single gene specific primer and the common primer occurs. Commercial cDNA pools modified for use in RACE are available.

Another PCR-based method generates full-length cDNA library with anchored ends without specific knowledge of the cDNA sequence. The method uses lock-docking primers (I-VI), where one primer, poly TV (I-III) locks over the polyA tail of eukaryotic mRNA producing first strand synthesis and a second primer, polyGH (IV-VI) locks onto the polyC tail added by terminal deoxynucleotidyl transferase (TdT). This method is described in PCT Pub. No. WO 96/40998.

The promoter region of a gene generally is located 5' to the initiation site for RNA polymerase II. Hundreds of promoter regions contain the "TATA" box, a sequence such as TATTA or TATAA, which is sensitive to mutations. The promoter region can be obtained by performing 5' RACE using a primer from the coding region of the gene. Alternatively, the cDNA can be used as a probe for the genomic sequence, and the region 5' to the coding region is identified by "walking up."

If the gene is highly expressed or differentially expressed, the promoter from the gene may be of use in a regulatory construct for a heterologous gene.

Once the full-length cDNA or gene is obtained, DNA encoding variants can be prepared by site-directed mutagenesis, described in detail in Sambrook *et al.*, 15.3-15.63. The choice of codon or nucleotide to be replaced can be based on the disclosure herein on optional changes in amino acids to achieve altered protein structure and/or function.

As an alternative method to obtaining DNA or RNA from a biological material, nucleic acid comprising nucleotides having the sequence of one or more nucleic acids of the invention can be synthesized. Thus, the invention encompasses nucleic acid molecules ranging in length from 12 nucleotides (corresponding to at least 12 contiguous nucleotides which hybridize under stringent conditions to or are at least 80% identical to a nucleic acid represented by one of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto) up to a maximum length suitable for one or more biological manipulations, including replication and expression, of the nucleic acid molecule. The invention includes but is not limited to (a) nucleic acid having the size of a full gene, and comprising at least one of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto; (b) the nucleic acid of (a) also comprising at least one additional gene, operably linked to permit expression of a fusion protein; (c) an expression vector comprising (a) or (b); (d) a plasmid comprising (a) or (b); and (e) a recombinant viral particle comprising (a) or (b). Construction of (a) can be accomplished as described below in part IV.

The sequence of a nucleic acid of the present invention is not limited and can be any sequence of A, T, G, and/or C (for DNA) and A, U, G, and/or C (for RNA) or modified bases thereof, including inosine and pseudouridine. The choice of sequence will depend on the desired function and can be dictated by coding regions desired, the intron-like regions desired, and the regulatory regions desired.

VI. Vectors Carrying Nucleic Acids of the Present Invention

The invention further provides plasmids and vectors, which can be used to express a gene in a host cell. The host cell may be any prokaryotic or eukaryotic cell. Thus, a nucleotide sequence derived from any one of SEQ ID Nos. 1-850, preferably

SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, encoding all or a selected portion of a protein, can be used to produce a recombinant form of an polypeptide via microbial or eukaryotic cellular processes. Ligating the polynucleotide sequence into a gene construct, such as an expression vector, and transforming or transfecting into hosts, either eukaryotic (yeast, avian, insect or mammalian) or prokaryotic (bacterial cells), are standard procedures well known in the art.

Vectors that allow expression of a nucleic acid in a cell are referred to as expression vectors. Typically, expression vectors contain a nucleic acid operably linked to at least one transcriptional regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the subject nucleic acids. Transcriptional regulatory sequences are described in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). In one embodiment, the expression vector includes a recombinant gene encoding a peptide having an agonistic activity of a subject polypeptide, or alternatively, encoding a peptide which is an antagonistic form of a subject polypeptide.

The choice of plasmid will depend on the type of cell in which propagation is desired and the purpose of propagation. Certain vectors are useful for amplifying and making large amounts of the desired DNA sequence. Other vectors are suitable for expression in cells in culture. Still other vectors are suitable for transfer and expression in cells in a whole animal or person. The choice of appropriate vector is well within the skill of the art. Many such vectors are available commercially. The nucleic acid or full-length gene is inserted into a vector typically by means of DNA ligase attachment to a cleaved restriction enzyme site in the vector. Alternatively, the desired nucleotide sequence may be inserted by homologous recombination in vivo. Typically this is accomplished by attaching regions of homology to the vector on the flanks of the desired nucleotide sequence. Regions of homology are added by ligation of oligonucleotides, or by polymerase chain reaction using primers comprising both the region of homology and a portion of the desired nucleotide sequence, for example.

Nucleic acids or full-length genes are linked to regulatory sequences as appropriate to obtain the desired expression properties. These may include promoters (attached either at the 5' end of the sense strand or at the 3' end of the antisense

strand), enhancers, terminators, operators, repressors, and inducers. The promoters may be regulated or constitutive. In some situations it may be desirable to use conditionally active promoters, such as tissue-specific or developmental stage-specific promoters. These are linked to the desired nucleotide sequence using the techniques described above for linkage to vectors. Any techniques known in the art may be used.

When any of the above host cells, or other appropriate host cells or organisms, are used to replicate and/or express the polynucleotides or nucleic acids of the invention, the resulting replicated nucleic acid, RNA, expressed protein or polypeptide, is within the scope of the invention as a product of the host cell or organism. The product is recovered by any appropriate means known in the art.

Once the gene corresponding to the nucleic acid is identified, its expression can be regulated in the cell to which the gene is native. For example, an endogenous gene of a cell can be regulated by an exogenous regulatory sequence as disclosed in U.S. Patent No. 5,641,670, "Protein Production and Protein Delivery."

A number of vectors exist for the expression of recombinant proteins in yeast (see, for example, Broach *et al.* (1983) in Experimental Manipulation of Gene Expression, ed. M. Inouye, Academic Press, p. 83, incorporated by reference herein). In addition, drug resistance markers such as ampicillin can be used. In an illustrative embodiment, a polypeptide is produced recombinantly utilizing an expression vector generated by sub-cloning one of the nucleic acids represented in one of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto.

The preferred mammalian expression vectors contain both prokaryotic sequences, to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The various methods employed in the preparation of plasmids and transformation of host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see Molecular Cloning: A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989) Chapters 16 and 17.
When it is desirable to express only a portion of a gene, e.g., a truncation mutant, it may be necessary to add a start codon (ATG) to the oligonucleotide fragment

containing the desired sequence to be expressed. It is well known in the art that a methionine at the N-terminal position can be enzymatically cleaved by the use of the enzyme methionine aminopeptidase (MAP). MAP has been cloned from E. coli (Ben-Bassat *et al.* (1987) J. Bacteriol. 169:751-757) and Salmonella typhimurium and its *in vitro* activity has been demonstrated on recombinant proteins (Miller *et al.* (1987) PNAS 84:2718-1722). Therefore, removal of an N-terminal methionine, if desired, can be achieved either *in vivo* by expressing polypeptides in a host which produces MAP (e.g., E. coli or CM89 or S. cerevisiae), or *in vitro* by use of purified MAP (e.g., procedure of Miller *et al.*, *supra*).

Moreover, the nucleic acid constructs of the present invention can also be used as part of a gene therapy protocol to deliver nucleic acids such as antisense nucleic acids. Thus, another aspect of the invention features expression vectors for *in vivo* or *in vitro* transfection with an antisense oligonucleotide.

In addition to viral transfer methods, non-viral methods can also be employed to introduce a subject nucleic acid, e.g., a sequence represented by one of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, into the tissue of an animal. Most nonviral methods of gene transfer rely on normal mechanisms used by mammalian cells for the uptake and intracellular transport of macromolecules. In preferred embodiments, non-viral targeting means of the present invention rely on endocytic pathways for the uptake of the subject nucleic acid by the targeted cell. Exemplary targeting means of this type include liposomal derived systems, polylysine conjugates, and artificial viral envelopes.

A nucleic acid of any of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, the corresponding cDNA, or the full-length gene may be used to express the partial or complete gene product. Appropriate nucleic acid constructs are purified using standard recombinant DNA techniques as described in, for example, Sambrook *et al.*, (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed. (Cold Spring Harbor Press, Cold Spring Harbor, New York), and under current regulations described in United States Dept. of HHS, National Institute of Health (NIH) Guidelines for Recombinant DNA Research. The polypeptides encoded by the nucleic acid may be expressed in

any expression system, including, for example, bacterial, yeast, insect, amphibian and mammalian systems. Suitable vectors and host cells are described in U.S. Patent No. 5,654,173.

Bacteria. Expression systems in bacteria include those described in Chang *et al.*, *Nature* (1978) *275*:615, Goeddel *et al.*, *Nature* (1979) *281*:544, Goeddel *et al.*, *Nucleic Acids Res.* (1980) *8*:4057; EP 0 036,776, U.S. Patent No. 4,551,433, DeBoer *et al.*, *Proc. Natl. Acad. Sci. (USA)* (1983) *80*:2125, and Siebenlist *et al.*, *Cell* (1980) *20*:269.

Yeast. Expression systems in yeast include those described in Hinnen *et al.*, *Proc. Natl. Acad. Sci. (USA)* (1978) *75*:1929; Ito *et al.*, *J. Bacteriol.* (1983) *153*:163; Kurtz *et al.*, *Mol. Cell. Biol.* (1986) *6*:142; Kunze *et al.*, *J. Basic Microbiol.* (1985) *25*:141; Gleeson *et al.*, *J. Gen. Microbiol.* (1986) *132*:3459, Roggenkamp *et al.*, *Mol. Gen. Genet.* (1986) *202*:302) Das *et al.*, *J. Bacteriol.* (1984) *158*:1165; De Louvencourt *et al.*, *J. Bacteriol.* (1983) *154*:737, Van den Berg *et al.*, *Bio/Technology* (1990) *8*:135; Kunze *et al.*, *J. Basic Microbiol.* (1985) *25*:141; Cregg *et al.*, *Mol. Cell. Biol.* (1985) *5*:3376, U.S. Patent Nos. 4,837,148 and 4,929,555; Beach and Nurse, *Nature* (1981) *300*:706; Davidow *et al.*, *Curr. Genet.* (1985) *10*:380, Gaillardin *et al.*, *Curr. Genet.* (1985) *10*:49, Ballance *et al.*, *Biochem. Biophys. Res. Commun.* (1983) *112*:284289; Tilburn *et al.*, *Gene* (1983) *26*:205221, Yelton *et al.*, *Proc. Natl. Acad. Sci. (USA)* (1984) *81*:14701474, Kelly and Hynes, *EMBO J.* (1985) *4*:475479; EP 0 244,234, and WO 91/00357.

Insect Cells. Expression of heterologous genes in insects is accomplished as described in U.S. Patent No. 4,745,051, Friesen *et al.* (1986) "The Regulation of Baculovirus Gene Expression" in: The Molecular Biology Of Baculoviruses (W. Doerfler, ed.), EP 0 127,839, EP 0 155,476, and Vlak *et al.*, J. *Gen. Virol.* (1988) *69*:765776, Miller *et al.*, *Ann. Rev. Microbiol.* (1988) *42*:177, Carbonell *et al.*, *Gene* (1988) *73*:409, Maeda *et al.*, *Nature* (1985) *315*:592594, LebacqVerheyden *et al.*, *Mol. Cell. Biol.* (1988) *8*:3129; Smith *et al.*, *Proc. Natl. Acad. Sci. (USA)* (1985) *82*:8404, Miyajima *et al.*, *Gene* (1987) *58*:273; and Martin *et al.*, *DNA* (1988) *7*:99. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts are described in Luckow *et al.*, *Bio/Technology* (1988) *6*:4755, Miller

et al., Generic Engineering (Setlow, J.K. *et al.* eds.), Vol. 8 (Plenum Publishing, 1986), pp. 277279, and Maeda *et al.*, *Nature*, (1985) *315*:592-594.

Mammalian Cells. Mammalian expression is accomplished as described in Dijkema *et al.*, *EMBO J.* (1985) *4*:761, Gorman *et al.*, *Proc. Natl. Acad. Sci. (USA)* (1982) *79*:6777, Boshart *et al.*, *Cell* (1985) *41*:521 and U.S. Patent No. 4,399,216. Other features of mammalian expression are facilitated as described in Ham and Wallace, *Meth. Enz.* (1979) *58*:44, Barnes and Sato, *Anal. Biochem.* (1980) *102*:255, U.S. Patent Nos. 4,767,704, 4,657,866, 4,927,762, 4,560,655, WO 90/103430, WO 87/00195, and U.S. RE 30,985.

VII. Therapeutic Nucleic Acid Constructs

One aspect of the invention relates to the use of the isolated nucleic acid, e.g., SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, in antisense therapy. As used herein, antisense therapy refers to administration or *in situ* generation of oligonucleotide molecules or their derivatives which specifically hybridize (e.g., bind) under cellular conditions with the cellular mRNA and/or genomic DNA, thereby inhibiting transcription and/or translation of that gene. The binding may be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix. In general, antisense therapy refers to the range of techniques generally employed in the art, and includes any therapy which relies on specific binding to oligonucleotide sequences.

An antisense construct of the present invention can be delivered, for example, as an expression plasmid which, when transcribed in the cell, produces RNA which is complementary to at least a unique portion of the cellular mRNA. Alternatively, the antisense construct is an oligonucleotide probe which is generated *ex vivo* and which, when introduced into the cell, causes inhibition of expression by hybridizing with the mRNA and/or genomic sequences of a subject nucleic acid. Such oligonucleotide probes are preferably modified oligonucleotides which are resistant to endogenous nucleases, e.g., exonucleases and/or endonucleases, and are therefore stable *in vivo*. Exemplary nucleic acid molecules for use as antisense oligonucleotides are

phosphoramidate, phosphorothioate and methylphosphonate analogs of DNA (see also U.S. Patents 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy have been reviewed, for example, by Van der Krol et al. (1988) BioTechniques 6:958-976; and Stein et al. (1988) Cancer Res 48:2659-2668. With respect to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, e.g., between the -10 and +10 regions of the nucleotide sequence of interest, are preferred.

Antisense approaches involve the design of oligonucleotides (either DNA or RNA) that are complementary to mRNA. The antisense oligonucleotides will bind to the mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. In the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the mRNA, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have recently been shown to be effective at inhibiting translation of mRNAs as well. (Wagner, R. 1994. Nature 372:333). Therefore, oligonucleotides complementary to either the 5' or 3' untranslated, non-coding regions of a gene could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are typically less efficient inhibitors of translation but could also be used in accordance with the invention. Whether designed to hybridize to the 5', 3', or coding region of subject mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably

less that about 100 and more preferably less than about 50, 25, 17 or 10 nucleotides in length.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO 88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO 89/10134, published April 25, 1988), hybridization-triggered cleavage agents (See, e.g., Krol et al., 1988, BioTechniques 6:958-976), or intercalating agents (See, e.g., Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxytriethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-

carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

The antisense oligonucleotide can also contain a neutral peptide-like backbone. Such molecules are termed peptide nucleic acid (PNA)-oligomers and are described, e.g., in Perry-O'Keefe et al. (1996) Proc. Natl. Acad. Sci. U.S.A. 93:14670 and in Eglom *et al.* (1993) Nature 365:566. One advantage of PNA oligomers is their capability to bind to complementary DNA essentially independently from the ionic strength of the medium due to the neutral backbone of the DNA. In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet a further embodiment, the antisense oligonucleotide is an α-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-12148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

Oligonucleotides of the invention may be synthesized by standard methods known in the art, e.g., by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate olgonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

While antisense nucleotides complementary to a coding region sequence can be used, those complementary to the transcribed untranslated region and to the region comprising the initiating methionine are most preferred.

The antisense molecules can be delivered to cells which express the target nucleic acid *in vivo*. A number of methods have been developed for delivering antisense DNA or RNA to cells; e.g., antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

However, it is often difficult to achieve intracellular concentrations of the antisense sufficient to suppress translation on endogenous mRNAs. Therefore, a preferred approach utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous transcripts and thereby prevent translation of the target mRNA. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40

early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al, 1982, Nature 296:39-42), etc. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site; e.g., the choroid plexus or hypothalamus. Alternatively, viral vectors can be used which selectively infect the desired tissue (e.g., for brain, herpesvirus vectors may be used), in which case administration may be accomplished by another route (e.g., systemically).

In another aspect of the invention, ribozyme molecules designed to catalytically cleave target mRNA transcripts can be used to prevent translation of target mRNA and expression of a target protein (See, e.g., PCT International Publication WO90/11364, published October 4, 1990; Sarver *et al.*, 1990, Science 247:1222-1225 and U.S. Patent No. 5,093,246). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy target mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, 1988, Nature, 334:585-591. Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the target mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one which occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA) and which has been extensively described by Thomas Cech and collaborators (Zaug, et al., 1984, Science, 224:574-578; Zaug and Cech, 1986, Science, 231:470-475; Zaug, et al., 1986, Nature, 324:429-433; published International patent application No. WO88/04300 by University Patents Inc.; Been and Cech, 1986, Cell, 47:207-216). The Cech-type

ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes which target eight base-pair active site sequences that are present in a target gene.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.) and should be delivered to cells which express the target gene *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antisense RNA, DNA, and ribozyme molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Moreover, various well-known modifications to nucleic acid molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

VIII. Polypeptides of the Present Invention

The present invention makes available isolated polypeptides which are isolated from, or otherwise substantially free of other cellular proteins, especially other signal transduction factors and/or transcription factors which may normally be associated with the polypeptide. Subject polypeptides of the present invention include polypeptides encoded by the nucleic acids of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, or polypeptides encoded by genes of which a sequence in SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, is a fragment. Polypeptides of the present invention include those proteins which are differentially regulated in tumor cells, especially colon cancer-derived cell lines (relative to normal cells, e.g., normal colon tissue and non-colon tissue). In preferred embodiments, the polypeptides are upregulated in tumor cells, especially colon cancer cancer-derived cell lines. In other embodiments, the polypeptides are downregulated in tumor cells, especially colon cancer-derived cell lines. Proteins which are upregulated, such as oncogenes, or downregulated, such as tumor suppressors, in aberrantly proliferating cells may be targets for diagnostic or therapeutic techniques. For example, upregulation of the *cdc2* gene induces mitosis. Overexpression of the *myt1* gene, a mitotic deactivator, negatively regulates the activity of *cdc2*. Aberrant proliferation may thus be induced either by upregulating *cdc2* or by downregulating *myt1*

The term "substantially free of other cellular proteins" (also referred to herein as "contaminating proteins") or "substantially pure or purified preparations" are defined as encompassing preparations of polypeptides having less than about 20% (by dry weight) contaminating protein, and preferably having less than about 5% contaminating protein. Functional forms of the subject polypeptides can be prepared, for the first time, as purified preparations by using a cloned nucleic acid as described herein. Full length proteins or fragments corresponding to one or more particular motifs and/or domains or to arbitrary sizes, for example, at least about 5, 10, 25, 50, 75, or 100 amino acids in length are within the scope of the present invention.

For example, isolated polypeptides can be encoded by all or a portion of a nucleic acid sequence shown in any of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary

thereto. Isolated peptidyl portions of proteins can be obtained by screening peptides recombinantly produced from the corresponding fragment of the nucleic acid encoding such peptides. In addition, fragments can be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f-Moc or t-Boc chemistry. For example, a polypeptide of the present invention may be arbitrarily divided into fragments of desired length with no overlap of the fragments, or preferably divided into overlapping fragments of a desired length. The fragments can be produced (recombinantly or by chemical synthesis) and tested to identify those peptidyl fragments which can function as either agonists or antagonists of a wild-type (e.g., "authentic") protein.

Another aspect of the present invention concerns recombinant forms of the subject proteins. Recombinant polypeptides preferred by the present invention, in addition to native proteins as described above are encoded by a nucleic acid, which is at least 60%, more preferably at least 80%, and more preferably 85%, and more preferably 90%, and more preferably 95% identical to an amino acid sequence encoded by SEQ ID NOs. 1-850. Polypeptides which are encoded by a nucleic acid that is at least about 98-99% identical with the sequence of SEQ ID Nos. 1-850 are also within the scope of the invention. Also included in the present invention are peptide fragments comprising at least a portion of such a protein.

In a preferred embodiment, a polypeptide of the present invention is a mammalian polypeptide and even more preferably a human polypeptide. In particularly preferred embodiment, the polypeptide retains wild-type bioactivity. It will be understood that certain post-translational modifications, e.g., phosphorylation and the like, can increase the apparent molecular weight of the polypeptide relative to the unmodified polypeptide chain.

The present invention further pertains to recombinant forms of one of the subject polypeptides. Such recombinant polypeptides preferably are capable of functioning in one of either role of an agonist or antagonist of at least one biological activity of a wild-type ("authentic") polypeptide of the appended sequence listing. The term "evolutionarily related to", with respect to amino acid sequences of proteins, refers to both polypeptides having amino acid sequences which have arisen naturally,

and also to mutational variants of human polypeptides which are derived, for example, by combinatorial mutagenesis.

In general, polypeptides referred to herein as having an activity (e.g., are "bioactive") of a protein are defined as polypeptides which include an amino acid sequence encoded by all or a portion of the nucleic acid sequences shown in one of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, and which mimic or antagonize all or a portion of the biological/biochemical activities of a naturally occurring protein. According to the present invention, a polypeptide has biological activity if it is a specific agonist or antagonist of a naturally occurring form of a protein.

Assays for determining whether a compound, e.g, a protein or variant thereof, has one or more of the above biological activities are well known in the art. In certain embodiments, the polypeptides of the present invention have activities such as those outlined above.

In another embodiment, the coding sequences for the polypeptide can be incorporated as a part of a fusion gene including a nucleotide sequence encoding a different polypeptide. This type of expression system can be useful under conditions where it is desirable to produce an immunogenic fragment of a polypeptide (see, for example, EP Publication No: 0259149; and Evans *et al.* (1989) Nature 339:385; Huang *et al.* (1988) J. Virol. 62:3855; and Schlienger *et al.* (1992) J. Virol. 66:2). In addition to utilizing fusion proteins to enhance immunogenicity, it is widely appreciated that fusion proteins can also facilitate the expression of proteins, and, accordingly, can be used in the expression of the polypeptides of the present invention (see, for example, Current Protocols in Molecular Biology, eds. Ausubel et al. (N.Y.: John Wiley & Sons, 1991)). In another embodiment, a fusion gene coding for a purification leader sequence, such as a poly-(His)/enterokinase cleavage site sequence at the N-terminus of the desired portion of the recombinant protein, can allow purification of the expressed fusion protein by affinity chromatography using a Ni^{2+} metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the purified protein (e.g., see Hochuli *et al.* (1987) J. Chromatography 411:177; and Janknecht *et al.* PNAS 88:8972).

Techniques for making fusion genes are known to those skilled in the art. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of nucleic acid fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive nucleic acid fragments which can subsequently be annealed to generate a chimeric nucleic acid sequence (see, for example, Current Protocols in Molecular Biology, eds. Ausubel et al. John Wiley & Sons: 1992).

The present invention further pertains to methods of producing the subject polypeptides. For example, a host cell transfected with a nucleic acid vector directing expression of a nucleotide sequence encoding the subject polypeptides can be cultured under appropriate conditions to allow expression of the peptide to occur. Suitable media for cell culture are well known in the art. The recombinant polypeptide can be isolated from cell culture medium, host cells, or both using techniques known in the art for purifying proteins including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, and immunoaffinity purification with antibodies specific for such peptide. In a preferred embodiment, the recombinant polypeptide is a fusion protein containing a domain which facilitates its purification, such as GST fusion protein.

Moreover, it will be generally appreciated that, under certain circumstances, it may be advantageous to provide homologs of one of the subject polypeptides which function in a limited capacity as one of either an agonist (mimetic) or an antagonist, in order to promote or inhibit only a subset of the biological activities of the naturally occurring form of the protein. Thus, specific biological effects can be elicited by treatment with a homolog of limited function, and with fewer side effects relative to treatment with agonists or antagonists which are directed to all of the biological activities of naturally occurring forms of subject proteins.

Homologs of each of the subject polypeptide can be generated by mutagenesis, such as by discrete point mutation(s), or by truncation. For instance, mutation can give rise to homologs which retain substantially the same, or merely a subset, of the biological activity of the polypeptide from which it was derived. Alternatively, antagonistic forms of the polypeptide can be generated which are able to inhibit the function of the naturally occurring form of the protein, such as by competitively binding to a receptor.

The recombinant polypeptides of the present invention also include homologs of the wild-type proteins, such as versions of those proteins which are resistant to proteolytic cleavage, for example, due to mutations which alter ubiquitination or other enzymatic targeting associated with the protein.

Polypeptides may also be chemically modified to create derivatives by forming covalent or aggregate conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives of proteins can be prepared by linking the chemical moieties to functional groups on amino acid sidechains of the protein or at the N-terminus or at the C-terminus of the polypeptide.

Modification of the structure of the subject polypeptides can be for such purposes as enhancing therapeutic or prophylactic efficacy, stability (e.g., *ex vivo* shelf life and resistance to proteolytic degradation), or post-translational modifications (e.g., to alter phosphorylation pattern of protein). Such modified peptides, when designed to retain at least one activity of the naturally occurring form of the protein, or to produce specific antagonists thereof, are considered functional equivalents of the polypeptides described in more detail herein. Such modified peptides can be produced, for instance, by amino acid substitution, deletion, or addition. The substitutional variant may be a substituted conserved amino acid or a substituted non-conserved amino acid.

For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (i.e., isosteric and/or isoelectric mutations) will not have a major effect on the biological activity of the resulting molecule. Conservative replacements are those that

take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids can be divided into four families: (1) acidic = aspartate, glutamate; (2) basic = lysine, arginine, histidine; (3) nonpolar = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar = glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. In similar fashion, the amino acid repertoire can be grouped as (1) acidic = aspartate, glutamate; (2) basic = lysine, arginine histidine, (3) aliphatic = glycine, alanine, valine, leucine, isoleucine, serine, threonine, with serine and threonine optionally be grouped separately as aliphatic-hydroxyl; (4) aromatic = phenylalanine, tyrosine, tryptophan; (5) amide = asparagine, glutamine; and (6) sulfur -containing = cysteine and methionine. (see, for example, Biochemistry, 2nd ed., Ed. by L. Stryer, WH Freeman and Co.: 1981). Whether a change in the amino acid sequence of a peptide results in a functional homolog (e.g., functional in the sense that the resulting polypeptide mimics or antagonizes the wild-type form) can be readily determined by assessing the ability of the variant peptide to produce a response in cells in a fashion similar to the wild-type protein, or competitively inhibit such a response. Polypeptides in which more than one replacement has taken place can readily be tested in the same manner.The variant may be designed so as to retain biological activity of a particular region of the protein. In a non-limiting example, Osawa et al., 1994, Biochemistry and Molecular International 34:1003-1009, discusses the actin binding region of a protein from several different species. The actin binding regions of the these species are considered homologous based on the fact that they have amino acids that fall within "homologous residue groups." Homologous residues are judged according to the following groups (using single letter amino acid designations): STAG; ILVMF; HRK; DEQN; and FYW. For example, an S, a T, an A or a G can be in a position and the function (in this case actin binding) is retained.

Additional guidance on amino acid substitution is available from studies of protein evolution. Go et al., 1980, Int. J. Peptide Protein Res. 15:211-224, classified amino acid residue sites as interior or exterior depending on their accessibility. More frequent substitution on exterior sites was confirmed to be general in eight sets of homologous protein families regardless of their biological functions and the presence or absence of a prosthetic group. Virtually all types of amino acid residues had higher

mutabilities on the exterior than in the interior. No correlation between mutability and polarity was observed of amino acid residues in the interior and exterior, respectively. Amino acid residues were classified into one of three groups depending on their polarity: polar (Arg, Lys, His, Gln, Asn, Asp, and Glu); weak polar (Ala, Pro, Gly, Thr, and Ser), and nonpolar (Cys, Val, Met, Ile, Leu, Phe, Tyr, and Trp). Amino acid replacements during protein evolution were very conservative: 88% and 76% of them in the interior or exterior, respectively, were within the same group of the three. Inter-group replacements are such that weak polar residues are replaced more often by nonpolar residues in the interior and more often by polar residues on the exterior.

Querol et al., 1996, Prot. Eng. 9:265-271, provides general rules for amino acid substitutions to enhance protein thermostability. New glycosylation sites can be introduced as discussed in Olsen and Thomsen, 1991, J. Gen. Microbiol. 137:579-585. An additional disulfide bridge can be introduced, as discussed by Perry and Wetzel, 1984, Science 226:555-557; Pantoliano et al., 1987, Biochemistry 26:2077-2082; Matsumura et al., 1989, Nature 342:291-293; Nishikawa et al., 1990, Protein Eng. 3:443-448; Takagi et al., 1990, J. Biol. Chem. 265:6874-6878; Clarke et al., 1993, Biochemistry 32:4322-4329; and Wakarchuk et al., 1994, Protein Eng. 7:1379-1386.

An additional metal binding site can be introduced, according to Toma et al., 1991, Biochemistry 30:97-106, and Haezerbrouck et al., 1993, Protein Eng. 6:643-649. Substitutions with prolines in loops can be made according to Masul et al., 1994, Appl. Env. Microbiol. 60:3579-3584; and Hardy et al., FEBS Lett. 317:89-92.

Cysteine-depleted muteins are considered variants within the scope of the invention. These variants can be constructed according to methods disclosed in U.S. Patent No. 4,959,314, which discloses how to substitute other amino acids for cysteines, and how to determine biological activity and effect of the substitution. Such methods are suitable for proteins according to this invention that have cysteine residues suitable for such substitutions, for example to eliminate disulfide bond formation.

To learn the identity and function of the gene that correlates with an nucleic acid, the nucleic acids or corresponding amino acid sequences can be screened against profiles of protein families. Such profiles focus on common structural motifs among

proteins of each family. Publicly available profiles are described above. Additional or alternative profiles are described below.

In comparing a new nucleic acid with known sequences, several alignment tools are available. Examples include PileUp, which creates a multiple sequence alignment, and is described in Feng *et al.*, *J. Mol. Evol.* (1987) *25*:351-360. Another method, GAP, uses the alignment method of Needleman *et al.*, *J. Mol. Biol.* (1970) *48*:443-453. GAP is best suited for global alignment of sequences. A third method, BestFit, functions by inserting gaps to maximize the number of matches using the local homology algorithm of Smith and Waterman, *Adv. Appl. Math.* (1981) *2*:482-489.

Examples of such profiles are described below.

Chemokines

Chemokines are a family of proteins that have been implicated in lymphocyte trafficking, inflammatory diseases, angiogenesis, hematopoiesis, and viral infection. See, for example, Rollins, *Blood* (1997) *90(3)*:909-928, and Wells *et al.*, *J. Leuk. Biol.* (1997) *61*:545-550. U.S. Patent No. 5,605,817 discloses DNA encoding a chemokine expressed in fetal spleen. U.S. Patent No. 5,656,724 discloses chemokine-like proteins and methods of use. U.S. Patent No. 5,602,008 discloses DNA encoding a chemokine expressed by liver.

Mutants of the encoded chemokines are polypeptides having an amino acid sequence that possesses at least one amino acid substitution, addition, or deletion as compared to native chemokines. Fragments possess the same amino acid sequence of the native chemokines; mutants may lack the amino and/or carboxyl terminal sequences. Fusions are mutants, fragments, or the native chemokines that also include amino and/or carboxyl terminal amino acid extensions.

The number or type of the amino acid changes is not critical, nor is the length or number of the amino acid deletions, or amino acid extensions that are incorporated in the chemokines as compared to the native chemokine amino acid sequences. A polynucleotide encoding one of these variant polypeptides will retain at least about 80% amino acid identity with at least one known chemokine. Preferably, these polypeptides will retain at least about 85% amino acid sequence identity, more

preferably, at least about 90%; even more preferably, at least about 95%. In addition, the variants will exhibit at least 80%; preferably about 90%; more preferably about 95% of at least one activity exhibited by a native chemokine. Chemokine activity includes immunological, biological, receptor binding, and signal transduction functions of the native chemokine.

Chemotaxis. Assays for chemotaxis relating to neutrophils are described in Walz *et al.*, *Biochem. Biophys. Res. Commun.* (1987) *149*:755, Yoshimura *et al.*, *Proc. Natl. Acad. Sci. (USA)* (1987) *84*:9233, and Schroder *et al.*, *J. Immunol.* (1987) *139*:3474; to lymphocytes, Larsen *et al.*, *Science* (1989) *243*:1464, Carr *et al.*, *Proc. Natl. Acad. Sci. (USA)* (1994) *91*:3652; to tumor-infiltrating lymphocytes, Liao *et al.*, *J. Exp. Med* (1995). *182*:1301; to hemopoietic progenitors, Aiuti *et al.*, *J. Exp. Med.* (1997) *185*:111; to monocytes, Valente *et al.*, *Biochem.* (1988) *27*:4162; and to natural killer cells, Loetscher *et al.*, *J. Immunol.* (1996) *156*:322, and Allavena *et al.*, *Eur. J. Immunol.* (1994) *24*:3233.

Assays for determining the biological activity of attracting eosinophils are described in Dahinden *et al.*, *J. Exp. Med.* (1994) *179*:751, Weber *et al.*, *J. Immunol.* (1995) *154*:4166, and Noso *et al.*, *Biochem. Biophys. Res. Commun.* (1994) *200*:1470; for attracting dendritic cells, Sozzani *et al.*, *J. Immunol.* (1995) *155*:3292; for attracting basophils, in Dahinden *et al.*, *J. Exp. Med.* (1994) *179*:751, Alam *et al.*, *J. Immunol.* (1994) *152*:1298, Alam *et al.*, *J. Exp. Med.* (1992) *176*:781; and for activating neutrophils, Maghazaci *et al.*, *Eur. J. Immunol.* (1996) *26*:315, and Taub *et al.*, *J. Immunol.* (1995) *155*:3877. Native chemokines can act as mitogens for fibroblasts, assayed as described in Mullenbach *et al.*, *J. Biol. Chem.* (1986) *261*:719.

Receptor Binding. Native chemokines exhibit binding activity with a number of receptors. Description of such receptors and assays to detect binding are described in, for example, Murphy *et al.*, *Science* (1991) *253*:1280; Combadiere *et al.*, *J. Biol. Chem.* (1995) *270*:29671; Daugherty *et al.*, *J. Exp. Med.* (1996) *183*:2349; Samson *et al.*, *Biochem.* (1996) *35*:3362; Raport *et al.*, *J. Biol. Chem.* (1996) *271*:17161; Combadiere *et al.*, *J. Leukoc. Biol.* (1996) *60*:147; Baba *et al.*, *J. Biol. Chem.* (1997) *23*:14893; Yosida *et al.*, *J. Biol. Chem.* (1997) *272*:13803; Arvannitakis *et al.*, *Nature* (1997) *385*:347, and many other assays are known in the art.

Kinase Activiation. Assays for kinase activation are described by Yen *et al.*, *J. Leukoc. Biol.* (1997) *61*:529; Dubois *et al., J. Immunol.* (1996) *156*:1356; Turner *et al., J. Immunol.* (1995) *155*:2437. Assays for inhibition of angiogenesis or cell proliferation are described in Maione *et al., Science* (1990) *247*:77.

Glycosaminoglycan production can be induced by native chemokines, assayed as described in Castor *et al., Proc. Natl. Acad. Sci. (USA)* (1983) *80*:765. Chemokine-mediated histamine release from basophils is assayed as described in Dahinden *et al., J. Exp. Med.* (1989) *170*:1787; and White *et al., Immunol. Lett.* (1989) *22*:151. Heparin binding is described in Luster *et al., J. Exp. Med.* (1995) *182*:219.

Dimerization Activity. Chemokines can possess dimerization activity, which can be assayed according to Burrows *et al., Biochem.* (1994) *33*:12741; and Zhang *et al., Mol. Cell. Biol.* (1995) *15*:4851. Native chemokines can play a role in the inflammatory response of viruses. This activity can be assayed as described in Bleul *et al., Nature* (1996) *382*:829; and Oberlin *et al., Nature* (1996) *382*:833. Exocytosis of monocytes can be promoted by native chemokines. The assay for such activity is described in Uguccioni *et al., Eur. J. Immunol.* (1995) *25*:64. Native chemokines also can inhibit hemapoietic stem cell proliferation. The method for testing for such activity is reported in Graham *et al., Nature* (1990) *344*:442.

Death Domain Proteins

Several protein families contain death domain motifs (Feinstein and Kimchi, *TIBS Letters* (1995) *20*:242-244). Some death domain-containing proteins are implicated in cytotoxic intracellular signaling (Cleveland and Ihle, *Cell* (1995) *81*:479-482, Pan *et al, Science* (1997) *276*:111-113, Duan and Dixit, *Nature* (1997) *385*:86-89, and Chinnaiyan *et al, Science* (1996) *274*:990-992). U.S. Patent No. 5,563,039 describes a protein homologous to TRADD (Tumor Necrosis Factor Receptor-1 Associated Death Domain containing protein), and modifications of the active domain of TRADD that retain the functional characteristics of the protein, as well as apoptosis assays for testing the function of such death domain containing proteins. U.S. Patent No. 5,658,883 discloses biologically active TGF-B1 peptides. U.S. Patent No. 5,674,734 discloses protein RIP which contains a C-terminal death domain and an N-terminal kinase domain.

Leukemia Inhibitory Factor (LIF)

An LIF profile is constructed from sequences of leukemia inhibitor factor, CT-1 (cardiotrophin-1), CNTF (ciliary neurotrophic factor), OSM (oncostatin M), and IL-6 (interleukin-6). This profile encompasses a family of secreted cytokines that have pleiotropic effects on many cell types including hepatocytes, osteoclasts, neuronal cells and cardiac myocytes, and can be used to detect additional genes encoding such proteins. These molecules are all structurally related and share a common co-receptor gp130 which mediates intracellular signal transduction by cytoplasmic tyrosine kinases such as src.

Novel proteins related to this family are also likely to be secreted, to activate gp130 and to function in the development of a variety of cell types. Thus new members of this family would be candidates to be developed as growth or survival factors for the cell types that they stimulate. For more details on this family of cytokines, see Pennica *et al*, *Cytokine and Growth Factor Reviews* (1996) 7:81-91. U.S. Patent No. 5,420,247 discloses LIF receptor and fusion proteins. U.S. Patent No. 5,443,825 discloses human LIF.

Angiopoietin

Angiopoietin-1 is a secreted ligand of the TIE-2 tyrosine kinase; it functions as an angiogenic factor critical for normal vascular development. Angiopoietin-2 is a natural antagonist of angiopoietin-1 and thus functions as an anti-angiogenic factor. These two proteins are structurally similar and activate the same receptor. (Folkman and D'Amore, *Cell* (1996) *87*:1153-1155, and Davis *et al.*, *Cell* (1996) *87*:1161-1169.)

The angiopoietin molecules are composed of two domains, a coiled-coil region and a region related to fibrinogen. The fibrinogen domain is found in many molecules including ficolin and tesascin, and is well defined structurally with many members.

Receptor Protein-Tyrosine Kinases

Receptor Protein-Tyrosine Kinases or RPTKs are described in Lindberg, *Annu. Rev. Cell Biol.* (1994) *10*:251-337.

Growth Factors: Epidermal Growth Factor (EGF) and Fibroblast Growth Factor (FGF)

For a discussion of growth factor superfamilies, see Growth Factors: A Practical Approach, Appendix A1 (Ed. McKay and Leigh, Oxford University Press, NY, 1993) pp. 237-243.

The alignments (pretty box) for EGF and FGF are shown in Figures 1 and 2, respectively. U.S. Patent No. 4,444,760 discloses acidic brain fibroblast growth factor, which is active in the promotion of cell division and wound healing. U.S. Patent No. 5,439,818 discloses DNA encoding human recombinant basic fibroblast growth factor, which is active in wound healing. U.S. Patent No. 5,604,293 discloses recombinant human basic fibroblast growth factor, which is useful for wound healing. U.S. Patent No. 5,410,832 discloses brain-derived and recombinant acidic fibroblast growth factor, which act as mitogens for mesoderm and neuroectoderm-derived cells in culture, and promote wound healing in soft tissue, cartilaginous tissue and musculo-skeletal tissue. U.S. Patent No. 5,387,673 discloses biologically active fragments of FGF that retain activity.

Proteins of the TNF Family

A profile derived from the TNF family is created by aligning sequences of the following TNF family members: nerve growth factor (NGF), lymphotoxin, Fas ligand, tumor necrosis factor (TNF), CD40 ligand, TRAIL, ox40 ligand, 4-1BB ligand, CD27 ligand, and CD30 ligand. The profile is designed to identify sequences of proteins that constitute new members or homologues of this family of proteins.

U.S. Patent No. 5,606,023 discloses mutant TNF proteins; U.S. Patent No. 5,597,899 and U.S. Patent No. 5,486,463 disclose TNF muteins; and U.S. Patent No. 5,652,353 discloses DNA encoding TNFα muteins.

Members of the TNF family of proteins have been show in vitro to multimerize, as described in Burrows *et al.*, *Biochem.* (1994) *33*:12741 and Zhang *et al.*, *Mol. Cell. Biol.* (1995) 154851 and bind receptors as described in Browning *et al.*, *J. Immunol.* (1994) *147*:1230, Androlewicz *et al.*, *J. Biol. Chem.*(1992) *267*:2542, and Crowe *et al.*, *Science* (1994) *264*:707.

In vivo, TNFs proteolytically cleave a target protein as described in Kriegel *et al.*, *Cell* (1988) *53*:45 and Mohler *et al.*, *Nature* (1994) *370*:218 and demonstrate cell proliferation and differentiation activity. T-cell or thymocyte proliferation is assayed as described in Armitage *et al.*, *Eur. J. Immunol.* (1992) *22*:447; Current Protocols in Immunology, ed. J.E. Coligan *et al.*, 3.1-3.19; Takai *et al.*, *J. Immunol.* (1986) *137*:3494-3500, Bertagnoli *et al.*, *J. Immunol.* (1990) *145*:1706-1712, Bertagnoli *et al.*, *J. Immunol.* (1991) *133*:327-340, Bertagnoli *et al.*, *J. Immunol.* (1992) *149*:3778-3783, and Bowman *et al.*, *J. Immunol.* (1994) *152*:1756-1761. B cell proliferation and Ig secretion are assayed as described in Maliszewski, *J. Immunol.* (1990) *144*:3028-3033, and Assays for B Cell Function: In vitro antibody production, Mond and Brunswick, Current Protocols in Immunol., Coligan Ed vol 1 pp 3.8.1-3.8.16, John Wiley and Sons, Toronto 1994, Kehrl *et al.*, *Science* (1987) *238*:1144 and Boussiotis *et al.*, *PNAS USA* (1994) *91*:7007.

Other in vivo activities include upregulation of cell surface antigens, upregulation of costimulatory molecules, and cellular aggregation/adhesion as described in Barrett *et al.*, *J. Immunol.* (1991) *146*:1722; Bjorck *et al.*, *Eur. J. Immunol.* (1993) *23*:1771; Clark *et al.*, *Annu Rev. Immunol.* (1991) *9*:97; Ranheim *et al.*, *J. Exp. Med.* (1994) *177*:925; Yellin, *J. Immunol.* (1994) *153*:666; and Gruss *et al.*, *Blood* (1994) *84*:2305.

Proliferation and differentiation of hematopoietic and lymphopoietic cells has also been shown in vivo for TNFs, using assays for embryonic differentiation and hematopoiesis as described in Johansson *et al.*, *Cellular Biology* (1995) *15*:141-151, Keller *et al.*, *Mol. Cell. Biol.* (1993) *13*:473-486, McClanahan *et al.*, *Blood* (1993) *81*:2903-2915 and using assays to detect stem cell survival and differentiation as described in Culture of Hematopoietic Cells, Freshney *et al.* eds, pp 1-21, 23-29, 139-162, 163-179, and 265-268, Wiley-Liss, Inc., New York, NY, 1994, and Hirajama *et al.*, *PNAS USA* (1992) *89*:5907-5911.

In vivo activities of TNFs also include lymphocyte survival and apoptosis, assayed as described in Darzynkewicz *et al.*, *Cytometry* (1992) *13*:795-808; Gorczca *et al.*, *Leukemia* (1993) *7*:659-670; Itoh *et al.*, *Cell* (1991) *66*:233-243; Zacharduk, *J. Immunol.* (1990) *145*:4037-4045; Zamai *et al.*, *Cytometry* (1993) *14*:891-897; and Gorczyca *et al.*, *Int'l J. Oncol.* (1992) *1*:639-648.

Some members of the TNF family are cleaved from the cell surface; others remain membrane bound. The three-dimensional structure of TNF is discussed in Sprang and Eck, Tumor Necrosis Factors; *supra.*

TNF proteins include a transmembrane domain. The protein is cleaved into a shorter soluble version, as described in Kriegler *et al.*, *Cell* (1988) *53*:45-53, Perez *et al.*, *Cell* (1990) *63*:251-258, and Shaw *et al.*, *Cell* (1986) *46*:659-667. The transmembrane domain is between amino acid 46 and 77 and the cytoplasmic domain is between position 1 and 45 on the human form of TNFα. The 3-dimensional motifs of TNF include a sandwich of two pleated β sheets. Each sheet is composed of anti-parallel α strands. α Strands facing each other on opposite sites of the sandwich are connected by short polypeptide loops, as described in Van Ostade *et al.*, *Protein Engineering* (1994) *7(1)*:5-22, and Sprang *et al.*, Tumor Necrosis Factors; *supra.*

Residues of the TNF family proteins that are involved in the β sheet secondary structure have been identified as described in Van Ostade *et al.*, *Protein Engineering* (1994) *7(1)*:5-22, and Sprang *et al.*, Tumor Necrosis Factors; *supra.*

TNF receptors are disclosed in U.S. Patent No. 5,395,760. A profile derived from the TNF receptor family is created by aligning sequences of the TNF receptor family, including Apo1/Fas, TNFR I and II, death receptor3 (DR3), CD40, ox40, CD27, and CD30. Thus, the profile is designed to identify, from the nucleic acids of the invention, sequences of proteins that constitute new members or homologs of this family of proteins.

Tumor necrosis factor receptors exist in two forms in humans: p55 TNFR and p75 TNFR, both of which provide intracellular signals upon binding with a ligand. The extracellular domains of these receptor proteins are cysteine rich. The receptors can remain membrane bound, although some forms of the receptors are cleaved forming soluble receptors. The regulation, diagnostic, prognostic, and therapeutic value of soluble TNF receptors is discussed in Aderka, *Cytokine and Growth Factor Reviews*, (1996) 7(3):231-240.

PDGF Family

U.S. Patent No. 5,326,695 discloses platelet derived growth factor agonists; bioactive portions of PDGF-B are used as agonists. U.S. Patent No. 4,845,075

discloses biologically active B-chain homodimers, and also includes variants and derivatives of the PDGF-B chain. U.S. Patent No. 5,128,321 discloses PDGF analogs and methods of use. Proteins having the same bioactivity as PDGF are disclosed, including A and B chain proteins.

Kinase (Including MKK) Family

U.S. Patent No. 5,650,501 discloses serine/threonine kinase, associated with mitotic and meiotic cell division; the protein has a kinase domain in its N-terminal and 3 PEST regions in the C-terminus. U.S. Patent No. 5,605,825 discloses human PAK65, a serine protein kinase.

The foregoing discussion provides a few examples of the protein profiles that can be compared with the nucleic acids of the invention. One skilled in the art can use these and other protein profiles to identify the genes that correlate with the nucleic acids.

IX. Determining the Function of the Encoded Expression Products

Ribozymes, antisense constructs, dominant negative mutants, and triplex formation can be used to determine function of the expression product of an nucleic acid-related gene.

A. Ribozymes

Trans-cleaving catalytic RNAs (ribozymes) are RNA molecules possessing endoribonuclease activity. Ribozymes are specifically designed for a particular target, and the target message must contain a specific nucleotide sequence. They are engineered to cleave any RNA species site-specifically in the background of cellular RNA. The cleavage event renders the mRNA unstable and prevents protein expression. Importantly, ribozymes can be used to inhibit expression of a gene of unknown function for the purpose of determining its function in an in vitro or in vivo context, by detecting the phenotypic effect.

One commonly used ribozyme motif is the hammerhead, for which the substrate sequence requirements are minimal. Design of the hammerhead ribozyme is disclosed in Usman *et al., Current Opin. Struct. Biol.* (1996) *6*:527-533. Usman

also discusses the therapeutic uses of ribozymes. Ribozymes can also be prepared and used as described in Long *et al.*, *FASEB J.* (1993) *7*:25; Symons, *Ann. Rev. Biochem.* (1992) *61*:641; Perrotta *et al.*, *Biochem.* (1992) *31*:16-17; Ojwang *et al.*, *Proc. Natl. Acad. Sci. (USA)* (1992) *89*:10802-10806; and U.S. Patent No. 5,254,678. Ribozyme cleavage of HIV-I RNA is described in U.S. Patent No. 5,144,019; methods of cleaving RNA using ribozymes is described in U.S. Patent No. 5,116,742; and methods for increasing the specificity of ribozymes are described in U.S. Patent No. 5,225,337 and Koizumi *et al.*, *Nucleic Acid Res.* (1989) *17*:7059-7071. Preparation and use of ribozyme fragments in a hammerhead structure are also described by Koizumi *et al.*, *Nucleic Acids Res.* (1989) *17*:7059-7071. Preparation and use of ribozyme fragments in a hairpin structure are described by Chowrira and Burke, *Nucleic Acids Res.* (1992) *20*:2835. Ribozymes can also be made by rolling transcription as described in Daubendiek and Kool, *Nat. Biotechnol.* (1997) *15(3)*:273-277.

The hybridizing region of the ribozyme may be modified or may be prepared as a branched structure as described in Horn and Urdea, *Nucleic Acids Res.* (1989) *17*:6959-67. The basic structure of the ribozymes may also be chemically altered in ways familiar to those skilled in the art, and chemically synthesized ribozymes can be administered as synthetic oligonucleotide derivatives modified by monomeric units. In a therapeutic context, liposome mediated delivery of ribozymes improves cellular uptake, as described in Birikh *et al.*, *Eur. J. Biochem.* (1997) *245*:1-16.

Using the nucleic acid sequences of the invention and methods known in the art, ribozymes are designed to specifically bind and cut the corresponding mRNA species. Ribozymes thus provide a means to inhibit the expression of any of the proteins encoded by the disclosed nucleic acids or their full-length genes. The full-length gene need not be known in order to design and use specific inhibitory ribozymes. In the case of an nucleic acid or cDNA of unknown function, ribozymes corresponding to that nucleotide sequence can be tested in vitro for efficacy in cleaving the target transcript. Those ribozymes that effect cleavage in vitro are further tested in vivo. The ribozyme can also be used to generate an animal model for a disease, as described in Birikh *et al.*, *Eur. J. Biochem.* (1997) *245*:1-16. An effective ribozyme is used to determine the function of the gene of interest by blocking its

transcription and detecting a change in the cell. Where the gene is found to be a mediator in a disease, an effective ribozyme is designed and delivered in a gene therapy for blocking transcription and expression of the gene.

Therapeutic and functional genomic applications of ribozymes proceed beginning with knowledge of a portion of the coding sequence of the gene to be inhibited. Thus, for many genes, a partial nucleic acid sequence provides adequate sequence for constructing an effective ribozyme. A target cleavage site is selected in the target sequence, and a ribozyme is constructed based on the 5' and 3' nucleotide sequences that flank the cleavage site. Retroviral vectors are engineered to express monomeric and multimeric hammerhead ribozymes targeting the mRNA of the target coding sequence. These monomeric and multimeric ribozymes are tested in vitro for an ability to cleave the target mRNA. A cell line is stably transduced with the retroviral vectors expressing the ribozymes, and the transduction is confirmed by Northern blot analysis and reverse-transcription polymerase chain reaction (RT-PCR). The cells are screened for inactivation of the target mRNA by such indicators as reduction of expression of disease markers or reduction of the gene product of the target mRNA.

B. Antisense

Antisense nucleic acids are designed to specifically bind to RNA, resulting in the formation of RNA-DNA or RNA-RNA hybrids, with an arrest of DNA replication, reverse transcription or messenger RNA translation. Antisense polynucleotides based on a selected nucleic acid sequence can interfere with expression of the corresponding gene. Antisense polynucleotides are typically generated within the cell by expression from antisense constructs that contain the antisense nucleic acid strand as the transcribed strand. Antisense nucleic acids will bind and/or interfere with the translation of nucleic acid-related mRNA. The expression products of control cells and cells treated with the antisense construct are compared to detect the protein product of the gene corresponding to the nucleic acid. The protein is isolated and identified using routine biochemical methods.

One rationale for using antisense methods to determine the function of the gene corresponding to an nucleic acid is the biological activity of antisense

therapeutics. Antisense therapy for a variety of cancers is in clinical phase and has been discussed extensively in the literature. Reed reviewed antisense therapy directed at the Bcl-2 gene in tumors; gene transfer-mediated overexpression of Bcl-2 in tumor cell lines conferred resistance to many types of cancer drugs. (Reed, J.C., *N.C.I.* (1997) *89*:988-990). The potential for clinical development of antisense inhibitors of *ras* is discussed by Cowsert, L.M., *Anti-Cancer Drug Design* (1997) *12*:359-371. Additional important antisense targets include leukemia (Geurtz, A.M., *Anti-Cancer Drug Design* (1997) *12*:341-358); human C-ref kinase (Monia, B.P., *Anti-Cancer Drug Design* (1997) *12*:327-339); and protein kinase C (McGraw *et al., Anti-Cancer Drug Design* (1997) *12*:315-326.

Given the extensive background literature and clinical experience in antisense therapy, one skilled in the art can use selected nucleic acids of the invention as additional potential therapeutics. The choice of nucleic acid can be narrowed by first testing them for binding to "hot spot" regions of the genome of cancerous cells. If an nucleic acid is identified as binding to a "hot spot", testing the nucleic acid as an antisense compound in the corresponding cancer cells clearly is warranted.

Ogunbiyi *et al.*, *Gastroenterology* (1997) *113(3)*:761-766 describe prognostic use of allelic loss in colon cancer; Barks *et al.*, *Genes, Chromosomes, and Cancer* (1997) *19(4)*:278-285 describe increased chromosome copy number detected by FISH in malignant melanoma; Nishizake *et al.*, *Genes, Chromosomes, and Cancer* (1997) *19(4)*:267-272 describe genetic alterations in primary breast cancer and their metastases and direct comparison using modified comparative genome hybridization; and Elo *et al.*, *Cancer Research* (1997) *57(16)*:3356-3359 disclose that loss of heterozygosity at 16z24.1-q24.2 is significantly associated with metastatic and aggressive behavior of prostate cancer.

C. Dominant Negative Mutations

As an alternative method for identifying function of the nucleic acid-related gene, dominant negative mutations are readily generated for corresponding proteins that are active as homomultimers. A mutant polypeptide will interact with wild-type polypeptides (made from the other allele) and form a non-functional multimer. Thus, a mutation is in a substrate-binding domain, a catalytic domain, or a cellular

localization domain. Preferably, the mutant polypeptide will be overproduced. Point mutations are made that have such an effect. In addition, fusion of different polypeptides of various lengths to the terminus of a protein can yield dominant negative mutants. General strategies are available for making dominant negative mutants. See Herskowitz, *Nature* (1987) *329*:219-222. Such a technique can be used for creating a loss-of-function mutation, which is useful for determining the function of a protein.

D. Triplex Formation

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene or its promoter using targeted homologous recombination. (E.g., see Smithies *et al.*, 1985, Nature 317:230-234; Thomas & Capecchi, 1987, Cell 51:503-512; Thompson et al., 1989 Cell 5:313-321; each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional gene (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous gene (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express that gene *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the gene.

Alternatively, endogenous gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the target gene (i.e., the gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells in the body. (See generally, Helene, C. 1991, Anticancer Drug Des., 6(6):569-84; Helene, C., et al., 1992, Ann, N.Y. Accad. Sci., 660:27-36; and Maher, L.J., 1992, Bioassays 14(12):807-15).

Nucleic acid molecules to be used in triple helix formation for the inhibition of transcription are preferably single stranded and composed of deoxyribonucleotides. The base composition of these oligonucleotides should promote triple helix formation via Hoogsteen base-pairing rules, which generally require sizable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences may be pyrimidine-based, which will result in TAT and CGC triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide

base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen that are purine-rich, for example, containing a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in CGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizable stretch of either purines or pyrimidines to be present on one strand of a duplex.

Antisense RNA and DNA, ribozyme, and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Moreover, various well known modifications to nucleic acid molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

X. Diagnostic & Prognostic Assays and Drug Screening Methods

The present invention provides method for determining whether a subject is at risk for developing a disease or condition characterized by unwanted cell proliferation by detecting the disclosed biomarkers, i.e., the disclosed nucleic acid markers (SEQ ID Nos: 1-850) and/or polypeptide markers for colon cancer encoded thereby.

In clinical applications, human tissue samples can be screened for the presence and/or absence of the biomarkers identified herein. Such samples could consist of needle biopsy cores, surgical resection samples, lymph node tissue, or serum. For example, these methods include obtaining a biopsy, which is optionally fractionated by cryostat sectioning to enrich tumor cells to about 80% of the total cell population. In certain embodiments, nucleic acids extracted from these samples may be amplified using techniques well known in the art. The levels of selected markers detected would be compared with statistically valid groups of metastatic, non-metastatic malignant, benign, or normal colon tissue samples.

In one embodiment, the diagnostic method comprises determining whether a subject has an abnormal mRNA and/or protein level of the disclosed markers, such as by Northern blot analysis, reverse transcription-polymerase chain reaction (RT-PCR), *in situ* hybridization, immunoprecipitation, Western blot hybridization, or immunohistochemistry. According to the method, cells are obtained from a subject and the levels of the disclosed biomarkers, protein or mRNA level, is determined and compared to the level of these markers in a healthy subject. An abnormal level of the biomarker polypeptide or mRNA levels is likely to be indicative of cancer such as colon cancer.

Accordingly, in one aspect, the invention provides probes and primers that are specific to the unique nucleic acid markers disclosed herein. Accordingly, the nucleic acid probes comprise a nucleotide sequence at least 12 nucleotides in length, preferably at least 15 nucleotides, more preferably, 25 nucleotides, and most preferably at least 40 nucleotides, and up to all or nearly all of the coding sequence which is complementary to a portion of the coding sequence of a marker nucleic acid sequence, which nucleic acid sequence is represented by SEQ ID Nos: 1-850 or a sequence complementary thereto.

In one embodiment, the method comprises using a nucleic acid probe to determine the presence of cancerous cells in a tissue from a patient. Specifically, the method comprises:

1. providing a nucleic acid probe comprising a nucleotide sequence at least 12 nucleotides in length, preferably at least 15 nucleotides, more preferably, 25 nucleotides, and most preferably at least 40 nucleotides, and up to all or nearly all of the coding sequence which is complementary to a portion of the coding sequence of a nucleic acid sequence represented by SEQ ID Nos: 1-850 or a sequence complementary thereto and is differentially expressed in tumors cells, such as colon cancer cells;
2. obtaining a tissue sample from a patient potentially comprising cancerous cells;
3. providing a second tissue sample containing cells substantially all of which are non-cancerous;
4. contacting the nucleic acid probe under stringent conditions with RNA of each of said first and second tissue samples (e.g., in a Northern blot or in situ hybridization assay); and
5. comparing (a) the amount of hybridization of the probe with RNA of the first tissue sample, with (b) the amount of hybridization of the probe with RNA of the second tissue sample;

wherein a statistically significant difference in the amount of hybridization with the RNA of the first tissue sample as compared to the amount of hybridization with the RNA of the second tissue sample is indicative of the presence of cancerous cells in the first tissue sample.

In one aspect, the method comprises in situ hybridization with a probe derived from a given marker nucleic acid sequence, which nucleic acid sequence is represented by SEQ ID Nos: 1-850 or a sequence complementary thereto. The method comprises contacting the labeled hybridization probe with a sample of a given

type of tissue potentially containing cancerous or precancerous cells as well as normal cells, and determining whether the probe labels some cells of the given tissue type to a degree significantly different (e.g., by at least a factor of two, or at least a factor of five, or at least a factor of twenty, or at least a factor of fifty) than the degree to which it labels other cells of the same tissue type.

Also within the invention is a method of determining the phenotype of a test cell from a given human tissue, e.g., whether the cell is (a) normal, or (b) cancerous or precancerous, by contacting the mRNA of a test cell with a nucleic acid probe at least 12 nucleotides in length, preferably at least 15 nucleotides, more preferably at least 25 nucleotides, and most preferably at least 40 nucleotides, and up to all or nearly all of a sequence which is complementary to a portion of the coding sequence of a nucleic acid sequence represented by SEQ ID Nos: 1-850 or a sequence complementary thereto, and which is differentially expressed in tumor cells as compared to normal cells of the given tissue type; and determining the approximate amount of hybridization of the probe to the mRNA, an amount of hybridization either more or less than that seen with the mRNA of a normal cell of that tissue type being indicative that the test cell is cancerous or precancerous.

Alternatively, the above diagnostic assays may be carried out using antibodies to detect the protein product encoded by the marker nucleic acid sequence, which nucleic acid sequence is represented by SEQ ID Nos: 1-850 or a sequence complementary thereto. Accordingly, in one embodiment, the assay would include contacting the proteins of the test cell with an antibody specific for the gene product of a nucleic acid represented by SEQ ID Nos: 1-850 or a sequence complementary thereto, the marker nucleic acid being one which is expressed at a given control level in normal cells of the same tissue type as the test cell, and determining the approximate amount of immunocomplex formation by the antibody and the proteins of the test cell, wherein a statistically significant difference in the amount of the immunocomplex formed with the proteins of a test cell as compared to a normal cell of the same tissue type is an indication that the test cell is cancerous or precancerous.

Another such method includes the steps of: providing an antibody specific for the gene product of a marker nucleic acid sequence represented by SEQ ID Nos 1-850, the gene product being present in cancerous tissue of a given tissue type (e.g.,

colon tissue) at a level more or less than the level of the gene product in noncancerous tissue of the same tissue type; obtaining from a patient a first sample of tissue of the given tissue type, which sample potentially includes cancerous cells; providing a second sample of tissue of the same tissue type (which may be from the same patient or from a normal control, e.g. another individual or cultured cells), this second sample containing normal cells and essentially no cancerous cells; contacting the antibody with protein (which may be partially purified, in lysed but unfractionated cells, or in situ) of the first and second samples under conditions permitting immunocomplex formation between the antibody and the marker nucleic acid sequence product present in the samples; and comparing (a) the amount of immunocomplex formation in the first sample, with (b) the amount of immunocomplex formation in the second sample, wherein a statistically significant difference in the amount of immunocomplex formation in the first sample less as compared to the amount of immunocomplex formation in the second sample is indicative of the presence of cancerous cells in the first sample of tissue.

The subject invention further provides a method of determining whether a cell sample obtained from a subject possesses an abnormal amount of marker polypeptide which comprises (a) obtaining a cell sample from the subject, (b) quantitatively determining the amount of the marker polypeptide in the sample so obtained, and (c) comparing the amount of the marker polypeptide so determined with a known standard, so as to thereby determine whether the cell sample obtained from the subject possesses an abnormal amount of the marker polypeptide. Such marker polypeptides may be detected by immunohistochemical assays, dot-blot assays, ELISA and the like.

Immunoassays are commonly used to quantitate the levels of proteins in cell samples, and many other immunoassay techniques are known in the art. The invention is not limited to a particular assay procedure, and therefore is intended to include both homogeneous and heterogeneous procedures. Exemplary immunoassays which can be conducted according to the invention include fluorescence polarization immunoassay (FPIA), fluorescence immunoassay (FIA), enzyme immunoassay (EIA), nephelometric inhibition immunoassay (NIA), enzyme linked immunosorbent assay (ELISA), and radioimmunoassay (RIA). An indicator moiety, or label group, can be

attached to the subject antibodies and is selected so as to meet the needs of various uses of the method which are often dictated by the availability of assay equipment and compatible immunoassay procedures. General techniques to be used in performing the various immunoassays noted above are known to those of ordinary skill in the art.

In another embodiment, the level of the encoded product, i.e., the product encoded by SEQ ID Nos 1-850 or a sequence complementary thereto, in a biological fluid (e.g., blood or urine) of a patient may be determined as a way of monitoring the level of expression of the marker nucleic acid sequence in cells of that patient. Such a method would include the steps of obtaining a sample of a biological fluid from the patient, contacting the sample (or proteins from the sample) with an antibody specific for a encoded marker polypeptide, and determining the amount of immune complex formation by the antibody, with the amount of immune complex formation being indicative of the level of the marker encoded product in the sample. This determination is particularly instructive when compared to the amount of immune complex formation by the same antibody in a control sample taken from a normal individual or in one or more samples previously or subsequently obtained from the same person.

In another embodiment, the method can be used to determine the amount of marker polypeptide present in a cell, which in turn can be correlated with progression of a hyperproliferative disorder, e.g., colon cancer. The level of the marker polypeptide can be used predictively to evaluate whether a sample of cells contains cells which are, or are predisposed towards becoming, transformed cells. Moreover, the subject method can be used to assess the phenotype of cells which are known to be transformed, the phenotyping results being useful in planning a particular therapeutic regimen. For instance, very high levels of the marker polypeptide in sample cells is a powerful diagnostic and prognostic marker for a cancer, such as colon cancer. The observation of marker polypeptide level can be utilized in decisions regarding, e.g., the use of more aggressive therapies.

As set out above, one aspect of the present invention relates to diagnostic assays for determining, in the context of cells isolated from a patient, if the level of a marker polypeptide is significantly reduced in the sample cells. The term "significantly reduced " refers to a cell phenotype wherein the cell possesses a

reduced cellular amount of the marker polypeptide relative to a normal cell of similar tissue origin. For example, a cell may have less than about 50%, 25%, 10%, or 5% of the marker polypeptide that a normal control cell. In particular, the assay evaluates the level of marker polypeptide in the test cells, and, preferably, compares the measured level with marker polypeptide detected in at least one control cell, e.g., a normal cell and/or a transformed cell of known phenotype.

Of particular importance to the subject invention is the ability to quantitate the level of marker polypeptide as determined by the number of cells associated with a normal or abnormal marker polypeptide level. The number of cells with a particular marker polypeptide phenotype may then be correlated with patient prognosis. In one embodiment of the invention, the marker polypeptide phenotype of the lesion is determined as a percentage of cells in a biopsy which are found to have abnormally high/low levels of the marker polypeptide. Such expression may be detected by immunohistochemical assays, dot-blot assays, ELISA and the like.

Where tissue samples are employed, immunohistochemical staining may be used to determine the number of cells having the marker polypeptide phenotype. For such staining, a multiblock of tissue is taken from the biopsy or other tissue sample and subjected to proteolytic hydrolysis, employing such agents as protease K or pepsin. In certain embodiments, it may be desirable to isolate a nuclear fraction from the sample cells and detect the level of the marker polypeptide in the nuclear fraction.

The tissue samples are fixed by treatment with a reagent such as formalin, glutaraldehyde, methanol, or the like. The samples are then incubated with an antibody, preferably a monoclonal antibody, with binding specificity for the marker polypeptides. This antibody may be conjugated to a label for subsequent detection of binding. Samples are incubated for a time sufficient for formation of the immuno-complexes. Binding of the antibody is then detected by virtue of a label conjugated to this antibody. Where the antibody is unlabeled, a second labeled antibody may be employed, e.g., which is specific for the isotype of the anti-marker polypeptide antibody. Examples of labels which may be employed include radionuclides, fluorescers, chemiluminescers, enzymes and the like.

Where enzymes are employed, the substrate for the enzyme may be added to the samples to provide a colored or fluorescent product. Examples of suitable

enzymes for use in conjugates include horseradish peroxidase, alkaline phosphatase, malate dehydrogenase and the like. Where not commercially available, such antibody-enzyme conjugates are readily produced by techniques known to those skilled in the art.

In one embodiment, the assay is performed as a dot blot assay. The dot blot assay finds particular application where tissue samples are employed as it allows determination of the average amount of the marker polypeptide associated with a single cell by correlating the amount of marker polypeptide in a cell-free extract produced from a predetermined number of cells.

It is well established in the cancer literature that tumor cells of the same type (e.g., breast and/or colon tumor cells) may not show uniformly increased expression of individual oncogenes or uniformly decreased expression of individual tumor suppressor genes. There may also be varying levels of expression of a given marker gene even between cells of a given type of cancer, further emphasizing the need for reliance on a battery of tests rather than a single test. Accordingly, in one aspect, the invention provides for a battery of tests utilizing a number of probes of the invention, in order to improve the reliability and/or accuracy of the diagnostic test.

In one embodiment, the present invention also provides a method wherein nucleic acid probes are immobilized on a DNA chip in an organized array. Oligonucleotides can be bound to a solid support by a variety of processes, including lithography. For example a chip can hold up to 250,000 oligonucleotides (GeneChip, Affymetrix). These nucleic acid probes comprise a nucleotide sequence at least about 12 nucleotides in length, preferably at least about 15 nucleotides, more preferably at least about 25 nucleotides, and most preferably at least about 40 nucleotides, and up to all or nearly all of a sequence which is complementary to a portion of the coding sequence of a marker nucleic acid sequence represented by SEQ ID Nos: 1-850 and is differentially expressed in tumor cells, such as colon cancer cells. The present invention provides significant advantages over the available tests for various cancers, such as colon cancer, because it increases the reliability of the test by providing an array of nucleic acid markers on a single chip.

The method includes obtaining a biopsy, which is optionally fractionated by cryostat sectioning to enrich tumor cells to about 80% of the total cell population. The

DNA or RNA is then extracted, amplified, and analyzed with a DNA chip to determine the presence of absence of the marker nucleic acid sequences.

In one embodiment, the nucleic acid probes are spotted onto a substrate in a two-dimensional matrix or array. Samples of nucleic acids can be labeled and then hybridized to the probes. Double-stranded nucleic acids, comprising the labeled sample nucleic acids bound to probe nucleic acids, can be detected once the unbound portion of the sample is washed away.

The probe nucleic acids can be spotted on substrates including glass, nitrocellulose, etc. The probes can be bound to the substrate by either covalent bonds or by non-specific interactions, such as hydrophobic interactions. The sample nucleic acids can be labeled using radioactive labels, fluorophores, chromophores, etc.

Techniques for constructing arrays and methods of using these arrays are described in EP No. 0 799 897; PCT No. WO 97/29212; PCT No. WO 97/27317; EP No. 0 785 280; PCT No. WO 97/02357; U.S. Pat. No. 5,593,839; U.S. Pat. No. 5,578,832; EP No. 0 728 520; U.S. Pat. No. 5,599,695; EP No. 0 721 016; U.S. Pat. No. 5,556,752; PCT No. WO 95/22058; and U.S. Pat. No. 5,631,734.

Further, arrays can be used to examine differential expression of genes and can be used to determine gene function. For example, arrays of the instant nucleic acid sequences can be used to determine if any of the nucleic acid sequences are differentially expressed between normal cells and cancer cells, for example. High expression of a particular message in a cancer cell, which is not observed in a corresponding normal cell, can indicate a cancer specific protein.

In yet another embodiment, the invention contemplates using a panel of antibodies which are generated against the marker polypeptides of this invention, which polypeptides are encoded by SEQ ID Nos 1-850. Such a panel of antibodies may be used as a reliable diagnostic probe for colon cancer. The assay of the present invention comprises contacting a biopsy sample containing cells, e.g., colon cells, with a panel of antibodies to one or more of the encoded products to determine the presence or absence of the marker polypeptides.

The diagnostic methods of the subject invention may also be employed as follow-up to treatment, e.g., quantitation of the level of marker polypeptides may be

indicative of the effectiveness of current or previously employed cancer therapies as well as the effect of these therapies upon patient prognosis.

Accordingly, the present invention makes available diagnostic assays and reagents for detecting gain and/or loss of marker polypeptides from a cell in order to aid in the diagnosis and phenotyping of proliferative disorders arising from, for example, tumorigenic transformation of cells.

The diagnostic assays described above can be adapted to be used as prognostic assays, as well. Such an application takes advantage of the sensitivity of the assays of the invention to events which take place at characteristic stages in the progression of a tumor. For example, a given marker gene may be up- or downregulated at a very early stage, perhaps before the cell is irreversibly committed to developing into a malignancy, while another marker gene may be characteristically up or down regulated only at a much later stage. Such a method could involve the steps of contacting the mRNA of a test cell with a nucleic acid probe derived from a given marker nucleic acid which is expressed at different characteristic levels in cancerous or precancerous cells at different stages of tumor progression, and determining the approximate amount of hybridization of the probe to the mRNA of the cell, such amount being an indication of the level of expression of the gene in the cell, and thus an indication of the stage of tumor progression of the cell; alternatively, the assay can be carried out with an antibody specific for the gene product of the given marker nucleic acid, contacted with the proteins of the test cell. A battery of such tests will disclose not only the existence and location of a tumor, but also will allow the clinician to select the mode of treatment most appropriate for the tumor, and to predict the likelihood of success of that treatment.

The methods of the invention can also be used to follow the clinical course of a tumor. For example, the assay of the invention can be applied to a tissue sample from a patient; following treatment of the patient for the cancer, another tissue sample is taken and the test repeated. Successful treatment will result in either removal of all cells which demonstrate differential expression characteristic of the cancerous or precancerous cells, or a substantial increase in expression of the gene in those cells, perhaps approaching or even surpassing normal levels.

In yet another embodiment, the invention provides methods for determining whether a subject is at risk for developing a disease, such as a predisposition to develop cancer, for example colon cancer, associated with an aberrant activity of any one of the polypeptides encoded by nucleic acids of SEQ ID Nos: 1-850, wherein the aberrant activity of the polypeptide is characterized by detecting the presence or absence of a genetic lesion characterized by at least one of (i) an alteration affecting the integrity of a gene encoding a marker polypeptides, or (ii) the mis-expression of the encoding nucleic acid. To illustrate, such genetic lesions can be detected by ascertaining the existence of at least one of (i) a deletion of one or more nucleotides from the nucleic acid sequence, (ii) an addition of one or more nucleotides to the nucleic acid sequence, (iii) a substitution of one or more nucleotides of the nucleic acid sequence, (iv) a gross chromosomal rearrangement of the nucleic acid sequence, (v) a gross alteration in the level of a messenger RNA transcript of the nucleic acid sequence, (vii) aberrant modification of the nucleic acid sequence, such as of the methylation pattern of the genomic DNA, (vii) the presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene, (viii) a non-wild type level of the marker polypeptide, (ix) allelic loss of the gene, and/or (x) inappropriate post-translational modification of the marker polypeptide.

The present invention provides assay techniques for detecting lesions in the encoding nucleic acid sequence. These methods include, but are not limited to, methods involving sequence analysis, Southern blot hybridization, restriction enzyme site mapping, and methods involving detection of absence of nucleotide pairing between the nucleic acid to be analyzed and a probe.

Specific diseases or disorders, e.g., genetic diseases or disorders, are associated with specific allelic variants of polymorphic regions of certain genes, which do not necessarily encode a mutated protein. Thus, the presence of a specific allelic variant of a polymorphic region of a gene in a subject can render the subject susceptible to developing a specific disease or disorder. Polymorphic regions in genes, can be identified, by determining the nucleotide sequence of genes in populations of individuals. If a polymorphic region is identified, then the link with a specific disease can be determined by studying specific populations of individuals, e.g, individuals which developed a specific disease, such as colon cancer. A

polymorphic region can be located in any region of a gene, e.g., exons, in coding or non coding regions of exons, introns, and promoter region.

In an exemplary embodiment, there is provided a nucleic acid composition comprising a nucleic acid probe including a region of nucleotide sequence which is capable of hybridizing to a sense or antisense sequence of a gene or naturally occurring mutants thereof, or 5' or 3' flanking sequences or intronic sequences naturally associated with the subject genes or naturally occurring mutants thereof. The nucleic acid of a cell is rendered accessible for hybridization, the probe is contacted with the nucleic acid of the sample, and the hybridization of the probe to the sample nucleic acid is detected. Such techniques can be used to detect lesions or allelic variants at either the genomic or mRNA level, including deletions, substitutions, etc., as well as to determine mRNA transcript levels.

A preferred detection method is allele specific hybridization using probes overlapping the mutation or polymorphic site and having about 5, 10, 20, 25, or 30 nucleotides around the mutation or polymorphic region. In a preferred embodiment of the invention, several probes capable of hybridizing specifically to allelic variants are attached to a solid phase support, e.g., a "chip". Mutation detection analysis using these chips comprising oligonucleotides, also termed "DNA probe arrays" is described e.g., in Cronin et al. (1996) Human Mutation 7:244. In one embodiment, a chip comprises all the allelic variants of at least one polymorphic region of a gene. The solid phase support is then contacted with a test nucleic acid and hybridization to the specific probes is detected. Accordingly, the identity of numerous allelic variants of one or more genes can be identified in a simple hybridization experiment.

In certain embodiments, detection of the lesion comprises utilizing the probe/primer in a polymerase chain reaction (PCR) (see, e.g. U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligase chain reaction (LCR) (see, e.g., Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *PNAS* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the gene (see Abravaya et al. (1995) *Nuc Acid Res* 23:675-682). In a merely illustrative embodiment, the method includes the steps of (i) collecting a sample of cells from a patient, (ii) isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, (iii)

contacting the nucleic acid sample with one or more primers which specifically hybridize to a nucleic acid sequence under conditions such that hybridization and amplification of the nucleic acid (if present) occurs, and (iv) detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. et al., 1989, Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.*, 1988, Bio/Technology 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In a preferred embodiment of the subject assay, mutations in, or allelic variants, of a gene from a sample cell are identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

Another aspect of the invention is directed to the identification of agents capable of modulating the differentiation and proliferation of cells characterized by aberrant proliferation. In this regard, the invention provides assays for determining compounds that modulate the expression of the marker nucleic acids (SEQ ID Nos: 1-850) and/or alter for example, inhibit the bioactivity of the encoded polypeptide.

Several in vivo methods can be used to identify compounds that modulate expression of the marker nucleic acids (SEQ ID Nos: 1-850) and/or alter for example, inhibit the bioactivity of the encoded polypeptide.

Drug screening is performed by adding a test compound to a sample of cells, and monitoring the effect. A parallel sample which does not receive the test compound is also monitored as a control. The treated and untreated cells are then compared by any suitable phenotypic criteria, including but not limited to microscopic analysis, viability testing, ability to replicate, histological examination, the level of a particular RNA or polypeptide associated with the cells, the level of enzymatic activity expressed by the cells or cell lysates, and the ability of the cells to interact with other cells or compounds. Differences between treated and untreated cells indicates effects attributable to the test compound.

Desirable effects of a test compound include an effect on any phenotype that was conferred by the cancer-associated marker nucleic acid sequence. Examples include a test compound that limits the overabundance of mRNA, limits production of the encoded protein, or limits the functional effect of the protein. The effect of the test compound would be apparent when comparing results between treated and untreated cells.

The invention thus also encompasses methods of screening for agents which inhibit expression of the nucleic acid markers (SEQ ID Nos: 1-850) in vitro, comprising exposing a cell or tissue in which the marker nucleic acid mRNA is detectable in cultured cells to an agent in order to determine whether the agent is capable of inhibiting production of the mRNA; and determining the level of mRNA in the exposed cells or tissue, wherein a decrease in the level of the mRNA after exposure of the cell line to the agent is indicative of inhibition of the marker nucleic acid mRNA production.

Alternatively, the screening method may include in vitro screening of a cell or tissue in which marker protein is detectable in cultured cells to an agent suspected of inhibiting production of the marker protein; and determining the level of the marker protein in the cells or tissue, wherein a decrease in the level of marker protein after exposure of the cells or tissue to the agent is indicative of inhibition of marker protein production.

The invention also encompasses in vivo methods of screening for agents which inhibit expression of the marker nucleic acids, comprising exposing a mammal having tumor cells in which marker mRNA or protein is detectable to an agent

suspected of inhibiting production of marker mRNA or protein; and determining the level of marker mRNA or protein in tumor cells of the exposed mammal. A decrease in the level of marker mRNA or protein after exposure of the mammal to the agent is indicative of inhibition of marker nucleic acid expression.

Accordingly, the invention provides a method comprising incubating a cell expressing the marker nucleic acids (SEQ ID Nos: 1-850) with a test compound and measuring the mRNA or protein level. The invention further provides a method for quantitatively determining the level of expression of the marker nucleic acids in a cell population, and a method for determining whether an agent is capable of increasing or decreasing the level of expression of the marker nucleic acids in a cell population. The method for determining whether an agent is capable of increasing or decreasing the level of expression of the marker nucleic acids in a cell population comprises the steps of (a) preparing cell extracts from control and agent-treated cell populations, (b) isolating the marker polypeptides from the cell extracts, (c) quantifying (e.g., in parallel) the amount of an immunocomplex formed between the marker polypeptide and an antibody specific to said polypeptide. The marker polypeptides of this invention may also be quantified by assaying for its bioactivity. Agents that induce increased the marker nucleic acid expression may be identified by their ability to increase the amount of immunocomplex formed in the treated cell as compared with the amount of the immunocomplex formed in the control cell. In a similar manner, agents that decrease expression of the marker nucleic acid may be identified by their ability to decrease the amount of the immunocomplex formed in the treated cell extract as compared to the control cell.

mRNA levels can be determined by Northern blot hybridization. mRNA levels can also be determined by methods involving PCR. Other sensitive methods for measuring mRNA, which can be used in high throughput assays, e.g., a method using a DELFIA endpoint detection and quantification method, are described, e.g., in Webb and Hurskainen (1996) *Journal of Biomolecular Screening* 1:119. Marker protein levels can be determined by immunoprecipitations or immunohistochemistry using an antibody that specifically recognizes the protein product encoded by SEQ ID Nos: 1-850.

Agents that are identified as active in the drug screening assay are candidates to be tested for their capacity to block cell proliferation activity. These agents would be useful for treating a disorder involving aberrant growth of cells, especially colon cells.

A variety of assay formats will suffice and, in light of the present disclosure, those not expressly described herein will nevertheless be comprehended by one of ordinary skill in the art. For instance, the assay can be generated in many different formats, and include assays based on cell-free systems, e.g., purified proteins or cell lysates, as well as cell-based assays which utilize intact cells.

In many drug screening programs which test libraries of compounds and natural extracts, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays of the present invention which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins or with lysates, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity and/or bioavailability of the test compound can be generally ignored in the *in vitro* system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity with other proteins or changes in enzymatic properties of the molecular target.

A. Use of Nucleic Acids as Probes in Mapping and in Tissue Profiling

Probes

Polynucleotide probes as described above, e.g., comprising at least 12 contiguous nucleotides selected from the nucleotide sequence of an nucleic acid as shown in SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, are used for a variety of purposes, including identification of human chromosomes and determining transcription levels. Additional disclosure about preferred regions of the nucleic acid sequences is found in the accompanying tables.

The nucleotide probes are labeled, for example, with a radioactive, fluorescent, biotinylated, or chemiluminescent label, and detected by well known methods appropriate for the particular label selected. Protocols for hybridizing nucleotide probes to preparations of metaphase chromosomes are also well known in the art. A nucleotide probe will hybridize specifically to nucleotide sequences in the chromosome preparations which are complementary to the nucleotide sequence of the probe. A probe that hybridizes specifically to an nucleic acid should provide a detection signal at least 5-, 10-, or 20-fold higher than the background hybridization provided with other unrelated sequences.

In a non-limiting example, commercial programs are available for identifying regions of chromosomes commonly associated with disease, such as cancer. Nucleic acids of the invention can be used to probe these regions. For example, if, through profile searching, a nucleic acid is identified as corresponding to a gene encoding a kinase, its ability to bind to a cancer-related chromosomal region will suggest its role as a kinase in one or more stages of tumor cell development/growth. Although some experimentation would be required to elucidate the role, the nucleic acid constitutes a new material for isolating a specific protein that has potential for developing a cancer diagnostic or therapeutic.

Nucleotide probes are used to detect expression of a gene corresponding to the nucleic acid. For example, in Northern blots, mRNA is separated electrophoretically and contacted with a probe. A probe is detected as hybridizing to an mRNA species of a particular size. The amount of hybridization is quantitated to determine relative amounts of expression, for example under a particular condition. Probes are also used to detect products of amplification by polymerase chain reaction. The products of the reaction are hybridized to the probe and hybrids are detected. Probes are used for in situ hybridization to cells to detect expression. Probes can also be used in vivo for diagnostic detection of hybridizing sequences. Probes are typically labeled with a radioactive isotope. Other types of detectable labels may be used such as chromophores, fluorophores, and enzymes.

Expression of specific mRNA can vary in different cell types and can be tissue specific. This variation of mRNA levels in different cell types can be exploited with nucleic acid probe assays to determine tissue types. For example, PCR, branched

DNA probe assays, or blotting techniques utilizing nucleic acid probes substantially identical or complementary to nucleic acids of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, can determine the presence or absence of target cDNA or mRNA.

Examples of a nucleotide hybridization assay are described in Urdea *et al.*, PCT WO92/02526 and Urdea *et al.*, U.S. Patent No. 5,124,246, both incorporated herein by reference. The references describe an example of a sandwich nucleotide hybridization assay.

Alternatively, the Polymerase Chain Reaction (PCR) is another means for detecting small amounts of target nucleic acids, as described in Mullis *et al.*, *Meth. Enzymol.* (1987) *155*:335-350; U.S. Patent No. 4,683,195; and U.S. Patent No. 4,683,202, all incorporated herein by reference. Two primer polynucleotides nucleotides hybridize with the target nucleic acids and are used to prime the reaction. The primers may be composed of sequence within or 3' and 5' to the polynucleotides of the Sequence Listing. Alternatively, if the primers are 3' and 5' to these polynucleotides, they need not hybridize to them or the complements. A thermostable polymerase creates copies of target nucleic acids from the primers using the original target nucleic acids as a template. After a large amount of target nucleic acids is generated by the polymerase, it is detected by methods such as Southern blots. When using the Southern blot method, the labeled probe will hybridize to a polynucleotide of the Sequence Listing or complement.

Furthermore, mRNA or cDNA can be detected by traditional blotting techniques described in Sambrook *et al.*, "Molecular Cloning: A Laboratory Manual" (New York, Cold Spring Harbor Laboratory, 1989). mRNA or cDNA generated from mRNA using a polymerase enzyme can be purified and separated using gel electrophoresis. The nucleic acids on the gel are then blotted onto a solid support, such as nitrocellulose. The solid support is exposed to a labeled probe and then washed to remove any unhybridized probe. Next, the duplexes containing the labeled probe are detected. Typically, the probe is labeled with radioactivity.

Mapping

Nucleic acids of the present invention are used to identify a chromosome on which the corresponding gene resides. Using fluorescence in situ hybridization (FISH) on normal metaphase spreads, comparative genomic hybridization allows total genome assessment of changes in relative copy number of DNA sequences. See Schwartz and Samad, *Current Opinions in Biotechnology* (1994) *8*:70-74; Kallioniemi *et al.*, *Seminars in Cancer Biology* (1993) *4*:41-46; Valdes and Tagle, *Methods in Molecular Biology* (1997) *68*:1, Boultwood, ed., Human Press, Totowa, NJ.

Preparations of human metaphase chromosomes are prepared using standard cytogenetic techniques from human primary tissues or cell lines. Nucleotide probes comprising at least 12 contiguous nucleotides selected from the nucleotide sequence of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, are used to identify the corresponding chromosome. The nucleotide probes are labeled, for example, with a radioactive, fluorescent, biotinylated, or chemiluminescent label, and detected by well known methods appropriate for the particular label selected. Protocols for hybridizing nucleotide probes to preparations of metaphase chromosomes are also well known in the art. A nucleotide probe will hybridize specifically to nucleotide sequences in the chromosome preparations that are complementary to the nucleotide sequence of the probe. A probe that hybridizes specifically to a target gene provides a detection signal at least 5-, 10-, or 20-fold higher than the background hybridization provided with unrelated coding sequences.

Nucleic acids are mapped to particular chromosomes using, for example, radiation hybrids or chromosome-specific hybrid panels. See Leach *et al.*, *Advances in Genetics*, (1995) *33*:63-99; Walter *et al.*, *Nature Genetics* (1994) *7*:22-28; Walter and Goodfellow, *Trends in Genetics* (1992) *9*:352. Panels for radiation hybrid mapping are available from Research Genentics, Inc., Huntsville, Alabama, USA. Databases for markers using various panels are available via the world wide web at http:/F/shgc-www.stanford.edu; and other locations. The statistical program RHMAP can be used to construct a map based on the data from radiation hybridization with a measure of the relative likelihood of one order versus another. RHMAP is available via the world wide web at http://www.sph.umich.edu/group/statgen/software.

Such mapping can be useful in identifying the function of the target gene by its proximity to other genes with known function. Function can also be assigned to the target gene when particular syndromes or diseases map to the same chromosome.

Tissue Profiling

The nucleic acids of the present invention can be used to determine the tissue type from which a given sample is derived. For example, a metastatic lesion is identified by its developmental organ or tissue source by identifying the expression of a particular marker of that organ or tissue. If a nucleic acid is expressed only in a specific tissue type, and a metastatic lesion is found to express that nucleic acid, then the developmental source of the lesion has been identified. Expression of a particular nucleic acid is assayed by detection of either the corresponding mRNA or the protein product. Immunological methods, such as antibody staining, are used to detect a particular protein product. Hybridization methods may be used to detect particular mRNA species, including but not limited to in situ hybridization and Northern blotting.

Use of Polymorphisms

A nucleic acid will be useful in forensics, genetic analysis, mapping, and diagnostic applications if the corresponding region of a gene is polymorphic in the human population. A particular polymorphic form of the nucleic acid may be used to either identify a sample as deriving from a suspect or rule out the possibility that the sample derives from the suspect. Any means for detecting a polymorphism in a gene are used, including but not limited to electrophoresis of protein polymorphic variants, differential sensitivity to restriction enzyme cleavage, and hybridization to an allele-specific probe.

B. Use of Nucleic Acids and Encoded Polypeptides to Raise Antibodies

Expression products of a nucleic acid, the corresponding mRNA or cDNA, or the corresponding complete gene are prepared and used for raising antibodies for experimental, diagnostic, and therapeutic purposes. For nucleic acids to which a corresponding gene has not been assigned, this provides an additional method of

identifying the corresponding gene. The nucleic acid or related cDNA is expressed as described above, and antibodies are prepared. These antibodies are specific to an epitope on the encoded polypeptide, and can precipitate or bind to the corresponding native protein in a cell or tissue preparation or in a cell-free extract of an in vitro expression system.

Immunogens for raising antibodies are prepared by mixing the polypeptides encoded by the nucleic acids of the present invention with adjuvants. Alternatively, polypeptides are made as fusion proteins to larger immunogenic proteins. Polypeptides are also covalently linked to other larger immunogenic proteins, such as keyhole limpet hemocyanin. Immunogens are typically administered intradermally, subcutaneously, or intramuscularly. Immunogens are administered to experimental animals such as rabbits, sheep, and mice, to generate antibodies. Optionally, the animal spleen cells are isolated and fused with myeloma cells to form hybridomas which secrete monoclonal antibodies. Such methods are well known in the art. According to another method known in the art, the nucleic acid is administered directly, such as by intramuscular injection, and expressed in vivo. The expressed protein generates a variety of protein-specific immune responses, including production of antibodies, comparable to administration of the protein.

Preparations of polyclonal and monoclonal antibodies specific for nucleic acid-encoded proteins and polypeptides are made using standard methods known in the art. The antibodies specifically bind to epitopes present in the polypeptides encoded by a nucleic acid of SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto. In another embodiment, the antibodies specifically bind to epitopes present in a polypeptide encoded by SEQ ID Nos. 1-850. Typically, at least about 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, for example, at least about 15, 25, or 50 amino acids. A short sequence of a nucleic acid may then be unsuitable for use as an epitope to raise antibodies for identifying the corresponding novel protein, because of the potential for cross-reactivity with a known protein. However, the antibodies may be useful for other purposes, particularly if they identify common

structural features of a known protein and a novel polypeptide encoded by a nucleic acid of the invention.

Antibodies that specifically bind to human nucleic acid-encoded polypeptides should provide a detection signal at least about 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in Western blots or other immunochemical assays. Preferably, antibodies that specifically bind nucleic acid T-encoded polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate nucleic acid-encoded proteins from solution.

To test for the presence of serum antibodies to the nucleic acid-encoded polypeptide in a human population, human antibodies are purified by methods well known in the art. Preferably, the antibodies are affinity purified by passing antiserum over a column to which an nucleic acid-encoded protein, polypeptide, or fusion protein is bound. The bound antibodies can then be eluted from the column, for example using a buffer with a high salt concentration.

In addition to the antibodies discussed above, genetically engineered antibody derivatives are made, such as single chain antibodies.

Antibodies may be made by using standard protocols known in the art (See, for example, Antibodies: A Laboratory Manual ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)). A mammal, such as a mouse, hamster, or rabbit can be immunized with an immunogenic form of the peptide (e.g., a mammalian polypeptide or an antigenic fragment which is capable of eliciting an antibody response, or a fusion protein as described above).

In one aspect, this invention includes monoclonal antibodies that show a subject polypeptide is highly expressed in colorectal tissue or tumor tissue, especially colon cancer tissue or colon cancer-derived cell lines. Therefore, in one embodiment, this invention provides a diagnostic tool for the analysis of expression of a subject polypeptide in general, and in particular, as a diagnostic for colon cancer.

Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques well known in the art. An immunogenic portion of a protein can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen

to assess the levels of antibodies. In a preferred embodiment, the subject antibodies are immunospecific for antigenic determinants of a protein of a mammal, e.g., antigenic determinants of a protein encoded by one of SEQ ID Nos. 1-850 or closely related homologs (e.g., at least 90% identical, and more preferably at least 95% identical).

Following immunization of an animal with an antigenic preparation of a polypeptide, antisera can be obtained and, if desired, polyclonal antibodies isolated from the serum. To produce monoclonal antibodies, antibody-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Such techniques are well known in the art, and include, for example, the hybridoma technique (originally developed by Kohler and Milstein, (1975) Nature, 256: 495-497), the human B cell hybridoma technique (Kozbar *et al.*, (1983) Immunology Today, 4: 72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., (1985) Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. pp. 77-96). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with a polypeptide of the present invention and monoclonal antibodies isolated from a culture comprising such hybridoma cells.

The term antibody as used herein is intended to include fragments thereof which are also specifically reactive with one of the subject polypeptides. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, $F(ab)_2$ fragments can be generated by treating antibody with pepsin. The resulting $F(ab)_2$ fragment can be treated to reduce disulfide bridges to produce Fab fragments. The antibody of the present invention is further intended to include bispecific, single-chain, and chimeric and humanized molecules having affinity for a polypeptide conferred by at least one CDR region of the antibody. In preferred embodiments, the antibodies, the antibody further comprises a label attached thereto and able to be detected, (e.g., the label can be a radioisotope, fluorescent compound, chemiluminescent compound, enzyme, or enzyme co-factor).

Antibodies can be used, e.g., to monitor protein levels in an individual for determining, e.g., whether a subject has a disease or condition, such as colon cancer, associated with an aberrant protein level, or allowing determination of the efficacy of a given treatment regimen for an individual afflicted with such a disorder. The level of polypeptides may be measured from cells in bodily fluid, such as in blood samples.

Another application of antibodies of the present invention is in the immunological screening of cDNA libraries constructed in expression vectors such as gt11, gt18-23, ZAP, and ORF8. Messenger libraries of this type, having coding sequences inserted in the correct reading frame and orientation, can produce fusion proteins. For instance, gt11 will produce fusion proteins whose amino termini consist of ß-galactosidase amino acid sequences and whose carboxyl termini consist of a foreign polypeptide. Antigenic epitopes of a protein, e.g., other orthologs of a particular protein or other paralogs from the same species, can then be detected with antibodies, as, for example, reacting nitrocellulose filters lifted from infected plates with antibodies. Positive phage detected by this assay can then be isolated from the infected plate. Thus, the presence of homologs can be detected and cloned from other animals, as can alternate isoforms (including splicing variants) from humans.

In another embodiment, a panel of monoclonal antibodies may be used, wherein each of the epitope's involved functions are represented by a monoclonal antibody. Loss or perturbation of binding of a monoclonal antibody in the panel would be indicative of a mutational attention of the protein and thus of the corresponding gene.

C. Differential Expression

The present invention also provides a method to identify abnormal or diseased tissue in a human. For nucleic acids corresponding to profiles of protein families as described above, the choice of tissue may be dictated by the putative biological function. The expression of a gene corresponding to a specific nucleic acid is compared between a first tissue that is suspected of being diseased and a second, normal tissue of the human. The normal tissue is any tissue of the human, especially those that express the target gene including, but not limited to, brain, thymus, testis,

heart, prostate, placenta, spleen, small intestine, skeletal muscle, pancreas, and the mucosal lining of the colon.

The tissue suspected of being abnormal or diseased can be derived from a different tissue type of the human, but preferably it is derived from the same tissue type; for example an intestinal polyp or other abnormal growth should be compared with normal intestinal tissue. A difference between the target gene, mRNA, or protein in the two tissues which are compared, for example in molecular weight, amino acid or nucleotide sequence, or relative abundance, indicates a change in the gene, or a gene which regulates it, in the tissue of the human that was suspected of being diseased.

The target genes in the two tissues are compared by any means known in the art. For example, the two genes are sequenced, and the sequence of the gene in the tissue suspected of being diseased is compared with the gene sequence in the normal tissue. The target genes, or portions thereof, in the two tissues are amplified, for example using nucleotide primers based on the nucleotide sequence shown in the Sequence Listing, using the polymerase chain reaction. The amplified genes or portions of genes are hybridized to nucleotide probes selected from a corresponding nucleotide sequence shown SEQ ID No. 1-850. A difference in the nucleotide sequence of the target gene in the tissue suspected of being diseased compared with the normal nucleotide sequence suggests a role of the nucleic acid-encoded proteins in the disease, and provides a lead for preparing a therapeutic agent. The nucleotide probes are labeled by a variety of methods, such as radiolabeling, biotinylation, or labeling with fluorescent or chemiluminescent tags, and detected by standard methods known in the art.

Alternatively, target mRNA in the two tissues is compared. PolyA$^+$ RNA is isolated from the two tissues as is known in the art. For example, one of skill in the art can readily determine differences in the size or amount of target mRNA transcripts between the two tissues using Northern blots and nucleotide probes selected from the nucleotide sequence shown in the Sequence Listing. Increased or decreased expression of a target mRNA in a tissue sample suspected of being diseased, compared with the expression of the same target mRNA in a normal tissue, suggests

that the expressed protein has a role in the disease, and also provides a lead for preparing a therapeutic agent.

Any method for analyzing proteins is used to compare two nucleic acid-encoded proteins from matched samples. The sizes of the proteins in the two tissues are compared, for example, using antibodies of the present invention to detect nucleic acid-encoded proteins in Western blots of protein extracts from the two tissues. Other changes, such as expression levels and subcellular localization, can also be detected immunologically, using antibodies to the corresponding protein. A higher or lower level of nucleic acid-encoded protein expression in a tissue suspected of being diseased, compared with the same nucleic acid-encoded protein expression level in a normal tissue, is indicative that the expressed protein has a role in the disease, and provides another lead for preparing a therapeutic agent.

Similarly, comparison of gene sequences or of gene expression products, e.g., mRNA and protein, between a human tissue that is suspected of being diseased and a normal tissue of a human, are used to follow disease progression or remission in the human. Such comparisons of genes, mRNA, or protein are made as described above.

For example, increased or decreased expression of the target gene in the tissue suspected of being neoplastic can indicate the presence of neoplastic cells in the tissue. The degree of increased expression of the target gene in the neoplastic tissue relative to expression of the gene in normal tissue, or differences in the amount of increased expression of the target gene in the neoplastic tissue over time, is used to assess the progression of the neoplasia in that tissue or to monitor the response of the neoplastic tissue to a therapeutic protocol over time.

The expression pattern of any two cell types can be compared, such as low and high metastatic tumor cell lines, or cells from tissue which have and have not been exposed to a therapeutic agent. A genetic predisposition to disease in a human is detected by comparing an target gene, mRNA, or protein in a fetal tissue with a normal target gene, mRNA, or protein. Fetal tissues that are used for this purpose include, but are not limited to, amniotic fluid, chorionic villi, blood, and the blastomere of an in vitro-fertilized embryo. The comparable normal target gene is obtained from any tissue. The mRNA or protein is obtained from a normal tissue of a human in which the target gene is expressed. Differences such as alterations in the

nucleotide sequence or size of the fetal target gene or mRNA, or alterations in the molecular weight, amino acid sequence, or relative abundance of fetal target protein, can indicate a germline mutation in the target gene of the fetus, which indicates a genetic predisposition to disease.

D. Use of Nucleic Acids, and Encoded Polypeptides to Screen for Peptide Analogs and Antagonists

Polypeptides encoded by the instant nucleic acids, e.g., SEQ ID Nos. 1-850, preferably SEQ ID Nos. 1-383, even more preferably SEQ ID Nos. 1-127, or a sequence complementary thereto, and corresponding full length genes can be used to screen peptide libraries to identify binding partners, such as receptors, from among the encoded polypeptides.

A library of peptides may be synthesized following the methods disclosed in U.S. Pat. No. 5,010,175, and in PCT WO 91/17823. As described below in brief, one prepares a mixture of peptides, which is then screened to identify the peptides exhibiting the desired signal transduction and receptor binding activity. In the '175 method, a suitable peptide synthesis support (e.g., a resin) is coupled to a mixture of appropriately protected, activated amino acids. The concentration of each amino acid in the reaction mixture is balanced or adjusted in inverse proportion to its coupling reaction rate so that the product is an equimolar mixture of amino acids coupled to the starting resin. The bound amino acids are then deprotected, and reacted with another balanced amino acid mixture to form an equimolar mixture of all possible dipeptides. This process is repeated until a mixture of peptides of the desired length (*e.g.*, hexamers) is formed. Note that one need not include all amino acids in each step: one may include only one or two amino acids in some steps (*e.g.*, where it is known that a particular amino acid is essential in a given position), thus reducing the complexity of the mixture. After the synthesis of the peptide library is completed, the mixture of peptides is screened for binding to the selected polypeptide. The peptides are then tested for their ability to inhibit or enhance activity. Peptides exhibiting the desired activity are then isolated and sequenced.

The method described in WO 91/17823 is similar. However, instead of reacting the synthesis resin with a mixture of activated amino acids, the resin is

divided into twenty equal portions (or into a number of portions corresponding to the number of different amino acids to be added in that step), and each amino acid is coupled individually to its portion of resin. The resin portions are then combined, mixed, and again divided into a number of equal portions for reaction with the second amino acid. In this manner, each reaction may be easily driven to completion. Additionally, one may maintain separate "subpools" by treating portions in parallel, rather than combining all resins at each step. This simplifies the process of determining which peptides are responsible for any observed receptor binding or signal transduction activity.

In such cases, the subpools containing, *e.g.*, 1-2,000 candidates each are exposed to one or more polypeptides of the invention. Each subpool that produces a positive result is then resynthesized as a group of smaller subpools (sub-subpools) containing, *e.g.*, 20-100 candidates, and reassayed. Positive sub-subpools may be resynthesized as individual compounds, and assayed finally to determine the peptides that exhibit a high binding constant. These peptides can be tested for their ability to inhibit or enhance the native activity. The methods described in WO 91/7823 and U.S. Patent No. 5,194,392 (herein incorporated by reference) enable the preparation of such pools and subpools by automated techniques in parallel, such that all synthesis and resynthesis may be performed in a matter of days.

Peptide agonists or antagonists are screened using any available method, such as signal transduction, antibody binding, receptor binding, mitogenic assays, chemotaxis assays, etc. The methods described herein are presently preferred. The assay conditions ideally should resemble the conditions under which the native activity is exhibited *in vivo*, that is, under physiologic pH, temperature, and ionic strength. Suitable agonists or antagonists will exhibit strong inhibition or enhancement of the native activity at concentrations that do not cause toxic side effects in the subject. Agonists or antagonists that compete for binding to the native polypeptide may require concentrations equal to or greater than the native concentration, while inhibitors capable of binding irreversibly to the polypeptide may be added in concentrations on the order of the native concentration.

The end results of such screening and experimentation will be at least one novel polypeptide binding partner, such as a receptor, encoded by a nucleic acid of the

invention, and at least one peptide agonist or antagonist of the novel binding partner. Such agonists and antagonists can be used to modulate, enhance, or inhibit receptor function in cells to which the receptor is native, or in cells that possess the receptor as a result of genetic engineering. Further, if the novel receptor shares biologically important characteristics with a known receptor, information about agonist/antagonist binding may help in developing improved agonists/antagonists of the known receptor.

E. Pharmaceutical Compositions and Therapeutic Uses

Pharmaceutical compositions can comprise polypeptides, antibodies, or polynucleotides of the claimed invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgment of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins,

polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in *Remington's Pharmaceutical Sciences* (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

Delivery Methods

Once formulated, the nucleic acid compositions of the invention can be (1) administered directly to the subject; (2) delivered ex vivo, to cells derived from the subject; or (3) delivered in vitro for expression of recombinant proteins.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a tumor or lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Methods for the ex vivo delivery and reimplantation of transformed cells into a subject are known in the art and described in e.g., International Publication No. WO 93/14778. Examples of cells useful in ex vivo applications include, for example, stem cells, particularly hematopoetic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both ex vivo and in vitro applications can be accomplished by, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

Once a subject gene has been found to correlate with a proliferative disorder, such as neoplasia, dysplasia, and hyperplasia, the disorder may be amenable to treatment by administration of a therapeutic agent based on the nucleic acid or corresponding polypeptide.

Preparation of antisense polypeptides is discussed above. Neoplasias that are treated with the antisense composition include, but are not limited to, cervical cancers, melanomas, colorectal adenocarcinomas, Wilms' tumor, retinoblastoma, sarcomas, myosarcomas, lung carcinomas, leukemias, such as chronic myelogenous leukemia, promyelocytic leukemia, monocytic leukemia, and myeloid leukemia, and lymphomas, such as histiocytic lymphoma. Proliferative disorders that are treated with the therapeutic composition include disorders such as anhydric hereditary ectodermal dysplasia, congenital alveolar dysplasia, epithelial dysplasia of the cervix, fibrous dysplasia of bone, and mammary dysplasia. Hyperplasias, for example, endometrial, adrenal, breast, prostate, or thyroid hyperplasias or pseudoepitheliomatous hyperplasia of the skin, are treated with antisense therapeutic compositions. Even in disorders in which mutations in the corresponding gene are not implicated, downregulation or inhibition of nucleic acid-related gene expression can have therapeutic application. For example, decreasing nucleic acid-related gene expression can help to suppress tumors in which enhanced expression of the gene is implicated.

Both the dose of the antisense composition and the means of administration are determined based on the specific qualities of the therapeutic composition, the condition, age, and weight of the patient, the progression of the disease, and other relevant factors. Administration of the therapeutic antisense agents of the invention includes local or systemic administration, including injection, oral administration, particle gun or catheterized administration, and topical administration. Preferably, the therapeutic antisense composition contains an expression construct comprising a

promoter and a polynucleotide segment of at least about 12, 22, 25, 30, or 35 contiguous nucleotides of the antisense strand of a nucleic acid. Within the expression construct, the polynucleotide segment is located downstream from the promoter, and transcription of the polynucleotide segment initiates at the promoter.

Various methods are used to administer the therapeutic composition directly to a specific site in the body. For example, a small metastatic lesion is located and the therapeutic composition injected several times in several different locations within the body of tumor. Alternatively, arteries which serve a tumor are identified, and the therapeutic composition injected into such an artery, in order to deliver the composition directly into the tumor. A tumor that has a necrotic center is aspirated and the composition injected directly into the now empty center of the tumor. The antisense composition is directly administered to the surface of the tumor, for example, by topical application of the composition. X-ray imaging is used to assist in certain of the above delivery methods.

Receptor-mediated targeted delivery of therapeutic compositions containing an antisense polynucleotide, subgenomic polynucleotides, or antibodies to specific tissues is also used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis *et al.*, *Trends in Biotechnol.* (1993) *11*:202-205; Chiou *et al.*, (1994) Gene Therapeutics: Methods And Applications Of Direct Gene Transfer (J.A. Wolff, ed.); Wu & Wu, *J. Biol. Chem.* (1988) *263*:621-24; Wu *et al.*, *J. Biol. Chem.* (1994) *269*:542-46; Zenke *et al.*, *Proc. Natl. Acad. Sci. (USA)* (1990) *87*:3655-59; Wu *et al.*, *J. Biol. Chem.* (1991) *266*:338-42. Preferably, receptor-mediated targeted delivery of therapeutic compositions containing antibodies of the invention is used to deliver the antibodies to specific tissue.

Therapeutic compositions containing antisense subgenomic polynucleotides are administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 mg to about 2 mg, about 5 mg to about 500 mg, and about 20 mg to about 100 mg of DNA can also be used during a gene therapy protocol. Factors such as method of action and efficacy of transformation and expression are considerations which will affect the dosage required for ultimate efficacy of the antisense subgenomic nucleic acids. Where greater expression is desired over a larger

area of tissue, larger amounts of antisense subgenomic nucleic acids or the same amounts readministered in a successive protocol of administrations, or several administrations to different adjacent or close tissue portions of, for example, a tumor site, may be required to effect a positive therapeutic outcome. In all cases, routine experimentation in clinical trials will determine specific ranges for optimal therapeutic effect. A more complete description of gene therapy vectors, especially retroviral vectors, is contained in U.S. Serial No. 08/869,309, which is expressly incorporated herein, and in section F below.

For genes encoding polypeptides or proteins with anti-inflammatory activity, suitable use, doses, and administration are described in U.S. Patent No. 5,654,173, incorporated herein by reference. Therapeutic agents also include antibodies to proteins and polypeptides encoded by the subject nucleic acids, as described in U.S. Patent No. 5,654,173.

F. Gene Therapy

The therapeutic nucleic acids of the present invention may be utilized in gene delivery vehicles. The gene delivery vehicle may be of viral or non-viral origin (see generally, Jolly, *Cancer Gene Therapy* (1994) *1*:51-64; Kimura, *Human Gene Therpay* (1994) *5*:845-852; Connelly, *Human Gene Therapy* (1995) *1*:185-193; and Kaplitt, *Nature Genetics* (1994) *6*:148-153). Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches. Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

The present invention can employ recombinant retroviruses which are constructed to carry or express a selected nucleic acid molecule of interest. Retrovirus vectors that can be employed include those described in EP 0 415 731; WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; U.S. Patent No. 5, 219,740; WO 93/11230; WO 93/10218; Vile and Hart, *Cancer Res.* (1993) *53*:3860-3864; Vile and Hart, *Cancer Res.* (1993) *53*:962-967; Ram et al., *Cancer Res.* (1993) *53*:83-88; Takamiya et al., J. *Neurosci. Res.* (1992) *33*:493-503; Baba et al., *J. Neurosurg.*

(1993) *79*:729-735; U.S. Patent no. 4,777,127; GB Patent No. 2,200,651; and EP 0 345 242. Preferred recombinant retroviruses include those described in WO 91/02805.

Packaging cell lines suitable for use with the above-described retroviral vector constructs may be readily prepared (see PCT publications WO 95/30763 and WO 92/05266), and used to create producer cell lines (also termed vector cell lines) for the production of recombinant vector particles. Within particularly preferred embodiments of the invention, packaging cell lines are made from human (such as HT1080 cells) or mink parent cell lines, thereby allowing production of recombinant retroviruses that can survive inactivation in human serum.

The present invention also employs alphavirus-based vectors that can function as gene delivery vehicles. Such vectors can be constructed from a wide variety of alphaviruses, including, for example, Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532). Representative examples of such vector systems include those described in U.S. Patent Nos. 5,091,309; 5,217,879; and 5,185,440; and PCT Publication Nos. WO 92/10578; WO 94/21792; WO 95/27069; WO 95/27044; and WO 95/07994.

Gene delivery vehicles of the present invention can also employ parvovirus such as adeno-associated virus (AAV) vectors. Representative examples include the AAV vectors disclosed by Srivastava in WO 93/09239, Samulski et al., *J. Vir.* (1989) *63*:3822-3828; Mendelson et al., *Virol.* (1988) *166*:154-165; and Flotte et al., *PNAS* (1993) *90*:10613-10617.

Representative examples of adenoviral vectors include those described by Berkner, *Biotechniques* (1988) *6*:616-627; Rosenfeld et al., *Science* (1991) *252*:431-434; WO 93/19191; Kolls et al., *PNAS* (1994) *91*:215-219; Kass-Eisler et al., *PNAS* (1993) *90*:11498-11502; Guzman et al., *Circulation* (1993) *88*:2838-2848; Guzman et al., *Cir. Res.* (1993) *73*:1202-1207; Zabner et al., *Cell* (1993) *75*:207-216; Li et al., *Hum. Gene Ther.* (1993) *4*:403-409; Cailaud et al., *Eur. J. Neurosci.* (1993) *5*:1287-1291; Vincent et al., *Nat. Genet.* (1993) *5*:130-134; Jaffe et al., *Nat. Genet.* (1992) *1*:372-378; and Levrero et al., *Gene* (1991) *101*:195-202. Exemplary adenoviral gene

therapy vectors employable in this invention also include those described in WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655. Administration of DNA linked to killed adenovirus as described in Curiel, *Hum. Gene Ther.* (1992) *3*:147-154 may be employed.

Other gene delivery vehicles and methods may be employed, including polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example Curiel, *Hum. Gene Ther.* (1992) *3*:147-154; ligand linked DNA, for example see Wu, *J. Biol. Chem.* (1989) *264*:16985-16987; eukaryotic cell delivery vehicles cells, for example see U.S. Serial No. 08/240,030, filed May 9, 1994, and U.S. Serial No. 08/404,796; deposition of photopolymerized hydrogel materials; hand-held gene transfer particle gun, as described in U.S. Patent No. 5,149,655; ionizing radiation as described in U.S. Patent No. 5,206,152 and in WO92/11033; nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip, *Mol. Cell Biol.* (1994) *14*:2411-2418, and in Woffendin, *Proc. Natl. Acad. Sci.* (1994) *91*:1581-1585.

Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and U.S. Patent No. 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm. Liposomes that can act as gene delivery vehicles are described in U.S. Patent No. 5,422,120, PCT Nos. WO 95/13796, WO 94/23697, and WO 91/14445, and EP No. 0 524 968.

Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al.*, *Proc. Natl. Acad. Sci. USA* (1994) *91*(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. Patent No. 5,149,655; use of ionizing radiation for activating

transferred gene, as described in U.S. Patent No. 5,206,152 and PCT No. WO 92/11033.

G. Transgenic Animals

One aspect of the present invention relates to transgenic non-human animals having germline and/or somatic cells in which the biological activity of one or more genes are altered by a chromosomally incorporated transgene.

In a preferred embodiments, the transgene encodes a mutant protein, such as dominant negative protein which antagonizes at least a portion of the biological function of a wild-type protein.

Yet another preferred transgenic animal includes a transgene encoding an antisense transcript which, when transcribed from the transgene, hybridizes with a gene or a mRNA transcript thereof, and inhibits expression of the gene.

In one embodiment, the present invention provides a desired non-human animal or an animal (including human) cell which contains a predefined, specific and desired alteration rendering the non-human animal or animal cell predisposed to cancer. Specifically, the invention pertains to a genetically altered non-human animal (most preferably, a mouse), or a cell (either non-human animal or human) in culture, that is defective in at least one of two alleles of a tumor-suppressor gene. The inactivation of at least one of these tumor suppressor alleles results in an animal with a higher susceptibility to tumor induction or other proliferative or differentiative disorders, or disorders marked by aberrant signal transduction, e.g., from a cytokine or growth factor. A genetically altered mouse of this type is able to serve as a useful model for hereditary cancers and as a test animal for carcinogen studies. The invention additionally pertains to the use of such non-human animals or animal cells, and their progeny in research and medicine.

Furthermore, it is contemplated that cells of the transgenic animals of the present invention can include other transgenes, e.g., which alter the biological activity of a second tumor suppressor gene or an oncogene. For instance, the second transgene can functionally disrupt the biological activity of a second tumor suppressor gene, such as p53, p73, DCC, $p21^{cip1}$, $p27^{kip1}$, Rb, Mad or E2F. Alternatively, the second transgene can cause overexpression or loss of regulation of an oncogene, such

as ras, myc, a cdc25 phosphatase, Bcl-2, Bcl-6, a transforming growth factor, neu, int-3, polyoma virus middle T antigen, SV40 large T antigen, a papillomaviral E6 protein, a papillomaviral E7 protein, CDK4, or cyclin D1.

A preferred transgenic non-human animal of the present invention has germline and/or somatic cells in which one or more alleles of a gene are disrupted by a chromosomally incorporated transgene, wherein the transgene includes a marker sequence providing a detectable signal for identifying the presence of the transgene in cells of the transgenic animal, and replaces at least a portion of the gene or is inserted into the gene or disrupts expression of a wild-type protein.

Still another aspect of the present invention relates to methods for generating non-human animals and stem cells having a functionally disrupted endogenous gene. In a preferred embodiment, the method comprises the steps of:

(i) constructing a transgene construct including (a) a recombination region having at least a portion of the gene, which recombination region directs recombination of the transgene with the gene, and (b) a marker sequence which provides a detectable signal for identifying the presence of the transgene in a cell;

(ii) transfering the transgene into stem cells of a non-human animal;

(iii) selecting stem cells having a correctly targeted homologous recombination between the transgene and the gene;

(iv) transfering cells identified in step (iii) into a non-human blastocyst and implanting the resulting chimeric blastocyst into a non-human female; and

(v) collecting offspring harboring an endogenous gene allele having the correctly targeted recombination.

Yet another aspect of the invention provides a method for evaluating the carcinogenic potential of an agent by (i) contacting a transgenic animal of the present invention with a test agent, and (ii) comparing the number of transformed cells in a sample from the treated animal with the number of transformed cells in a sample from an untreated transgenic animal or transgenic animal treated with a control agent. The difference in the number of transformed cells in the treated animal, relative to the number of transformed cells in the absence of treatment with a control agent, indicates the carcinogenic potential of the test compound.

Another aspect of the invention provides a method of evaluating an anti-proliferative activity of a test compound. In preferred embodiments, the method includes contacting a transgenic animal of the present invention, or a sample of cells from such animal, with a test agent, and determining the number of transformed cells in a specimen from the transgenic animal or in the sample of cells. A statistically significant decrease in the number of transformed cells, relative to the number of transformed cells in the absence of the test agent, indicates the test compound is a potential anti-proliferative agent.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, *Molecular Cloning A Laboratory Manual*, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press:1989); *DNA Cloning*, Volumes I and II (D. N. Glover ed., 1985); *Oligonucleotide Synthesis* (M. J. Gait ed., 1984); Mullis *et al.* U.S. Patent No. 4,683,195; *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); *Transcription And Translation* (B. D. Hames & S. J. Higgins eds. 1984); *Culture Of Animal Cells* (R. I. Freshney, Alan R. Liss, Inc., 1987); *Immobilized Cells And Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); *Methods In Enzymology*, Vols. 154 and 155 (Wu et al. eds.), *Immunochemical Methods In Cell And Molecular Biology* (Mayer and Walker, eds., Academic Press, London, 1987); *Handbook Of Experimental Immunology*, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

As mentioned above, the sequences described herein are believed to have particular utility in regards to colon cancer. However, they may also be useful with other types of cancers and other disease states.

The present invention will now be illustrated by reference to the following examples which set forth particularly advantageous embodiments. However, it should

be noted that these embodiments are illustrative and are not to be construed as restricting the invention in any way.

XI. Examples

A. Identification of differentially expressed sequences in the SW480 library

Description of the SW480 library

SEQ ID NO 1-850 were derived from the SW480 library. The SW480 library is a normalized, subtracted cDNA library that was generated from the RNA derived from colon cancer cell line SW480 and normal human colon tissue. Human colorectal adenocarcinoma (cancer) cell line SW480; ATCC #CCL228 (Leibovitz et al., Cancer Research 36:4562-4569, 1976) was used to generate double-stranded cDNA that was subsequently used as the tester sample for the subtraction experiment. Poly A^+ RNA from normal human colon tissue (purchased from OriGene Technologies, Inc. Rockville, MD) was used was used to generate double-stranded cDNA that was used as the driver sample for the subtraction experiment.

The growth conditions of the driver and tester sources in this library were different as SW480 is a rapidly growing cell line and may have higher cellular metabolism. Therefore some of the differential expression in this library might be due to non-relevant growth effects of the two sources of tissue.

Construction of the SW480 library

Double-stranded cDNA was generated using the Clontech SMART PCR cDNA Synthesis Kit (purchased from Clontech Laboratories Inc, Palo Alto, CA) following the manufacturer's instructions. Subtraction hybridization steps were performed in accordance with the manufacturer's instructions for the Clontech PCR-Select kit (purchased from Clontech Laboratories Inc, Palo Alto, CA). The subtracted cDNAs were then directly inserted into a T/A cloning vector (TOPO TA Cloning Kit, Invitrogen Corporation, Carlsbad, CA) according to manufacturer's instructions, transformed into *E. coli*, and plated onto LB-amp plates, containing X-gal and IPTG. 1248 bacterial colonies were picked, transferred to LB-

amp broth and propagated. Plasmids were isolated using column chromatography (QIAprep 96 Turbo Miniprep Kits, Qiagen Corporation, Valencia, CA) on the QIAGEN Biorobot 9600.

Initial validation of differential expression

The inserts from subtracted clones were amplified by PCR and 10ul of the PCR reaction product was run on a 2.0% agarose gel for 2 hr at 100 volts. The gel was blotted onto a nylon membrane according to standard methods and hybridized as follows: 50 ng aliquots of the RSA1 cut SW480 and normal colon cDNA libraries were labeled with [α-^{32}P] dCTP by Prime-It RmT Random Primer labeling kit (Stratagene, La Jolla, CA). Nylon membranes containing the PCR amplified DNA from the SW480 library clones were hybridized to the labeled probes at 4 x 10^6 cpm/ml in Express hybridization buffer (Clonetech) at 68°C for approximately16 hours. The membranes were subjected to stringent washes (0.1 X SSC; 0.1% SDS) done at 68°C and were then exposed to phosphorimager screens. The screens were analyzed using Molecular Dynamics ImageQuant software. Clones that exhibited a stronger hybridization signal with the SW480 probe relative to the normal colon probe were deemed to be differentially expressed.

Validation of differential expression in colon cancer

To validate that the differentially expressed sequences found in this library were specific to colon cancer, the clones were screened with cDNAs prepared from a colon cancer specific library, Delaware (DE), and a normal tissue specific library Maryland (MD).

The DE library is specific for sequences expressed in colon cancer [proximal and distal Dukes' B, microsatellite instibility negative (MSI-)] but not expressed in normal tissues, including colon. This colon cancer tissue specific cDNA library, was made using pooled colon cancer cDNA as tester (tumor tissue cDNA pooled from eight patients with either proximal stage B MSI$^-$ or distal stage B MSI$^-$ cancers). The driver cDNA consisted a combination of cDNAs made from 50% normal colon tissue and a pool of peripheral blood leukocytes (PBL), and normal liver, spleen, lung, kidney, heart, small intestine, skeletal muscle, and prostate tissue cDNAs as the remaining 50% of the driver.

The MD library is specific for sequences expressed in normal tissue, but not expressed in proximal and distal Dukes' B, MSI- colon cancers. The tester cDNA in this case was made up of 50% normal colon tissue cDNA while the other 50% was made up of PBL, liver, spleen, lung, kidney, heart, small intestine, skeletal muscle, and prostate tissue cDNAs. The driver for this library was generated from pools of proximal stage B, MSI$^-$ and distal stage B, MSI$^-$ tumor tissue cDNAs obtained from eight cancer patients.

SW 480 clones that hybridized with the DE probe, but hybridized to a lesser degree (or not at all) to the MD probe were determined to be differentially expressed. This confirmation of differential expression is additional evidence that the up regulation of the individual clones is related to colon cancer.

Sequencing and analysis of differentially expressed clones

The nucleotide sequence of the inserts from clones shown to be differentially expressed was determined by single-pass sequencing from either the T7 or M13 promoter sites using fluorescently labeled dideoxynucleotides via the Sanger sequencing method. Sequences were analyzed according to methods described in the text (XI., Examples; B. Results of Public Database Search).

Each nucleic acid represents sequence from at least a partial mRNA transcript. The nucleic acids of the invention were assigned a sequence identification number (see attachments). The DNA sequences are provided in the attachments containing the sequences.

Of the 1248 colonies examined, 826 individual clones were found to be differentially expressed using the SW480 and normal colon probes. Of these, 681 were found to be differentially expressed using the DE and MD tissue probes. 145 clones that previously showed differential expression with the SW480 and normal colon probes did not show differential expression with the DE and MD probes. 363 of these clones contained known sequences, 213 contained ESTs, and 105 contained novel sequences. An examination of the known sequences revealed that many of the genes are involved in cellular metabolism.

An example of an experiment to identify differentially expressed clones is shown in the Figure, "Differential Expression Analysis". The inserts from subtracted clones were amplified, electrophoresed, and blotted on to membranes as described above. The gel was hybridized with RSA1 cut DE and MD cDNA probes as described above.

In the Figure, individual clones are designated by a number at the top of each lane; the blots are aligned so that the same clone is represented in the same vertical lane in both the upper ("Cancer Probe") and lower ("Normal Probe") blot. Lanes labeled "O" indicate clones that are overexpressed, i.e., show a darker, more prominent band in the upper blot ("Cancer Probe") relative to that observed, in the same lane, in the lower blot ("Normal Probe"). The Lane labeled "U" indicates a clone that is underexpressed, i.e., shows a darker, more prominent band in the lower blot ("Normal Probe") relative to that observed, in the same lane, in the upper blot ("Cancer Probe"). The lane labeled "M", indicates a clone that is marginally overexpressed in cancer and normal cells.

B. Results of Public Database searches

The nucleotide sequence of SEQ ID Nos. 1-850 were aligned with individual sequences that were publicly available. Genbank and divisions of GenBank, such as dbEST, CGAP, and Unigene were the primary databases used to perform the sequence similarity searches. The patent database, GENESEQ, was also utilized.

A total of 850 sequences were analyzed; most sequences were between 200 and 700 nucleotides in length. The sequences were first masked to identify vector-derived sequences, which were subsequently removed. The remaining sequence information was used to create the sequences listed in the Sequence Listing (SEQ ID Nos. 1-850). Each of these sequences was used as the query sequence to perform a Blast 2 search against the databases listed above. The Blast 2 search differs from the traditional Blast search in that it allows for the introduction of gaps in order to produce an optimal alignment of two sequences.

A proprietary algorithm was developed to utilize the output from the Blast 2 searches and categorize the sequences based upon high similarity (e value < 1e-40) or

identity to entries contained in the GenBank and dbEST databases. Three categories were created as follows: 1) matches to known human genes, 2) matches to human EST sequences, and 3) no significant match to either 1 or 2, and therefore a potentially novel human sequence.

Those skilled in the art will recognize, or be able to ascertain, using not more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such specific embodiments and equivalents are intended to be encompassed by the following claims.

All patents, published patent applications, and publications cited herein are incorporated by reference as if set forth fully herein.

Table 1

SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes	SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes
1	SW0006	O	O	47	SW0558	O	O
2	SW0019M13	O	O	48	SW0585T7	O	O
3	SW0025T7	O	O	49	SW0602T7	O	O
4	SW0026T7	O	O	50	SW0605T7	O	O
5	SW0044	O	O	51	SW0638M13	O	O
6	SW0071	O	O	52	SW0638T7	O	O
7	SW0081T7	O	O	53	SW0652T7	O	O
8	SW0106	O	O	54	SW0659	O	O
9	SW0116	O	O	55	SW0663T7	M	O
10	SW0124	O	O	56	SW0678T7	O	O
11	SW0142M13	O	O	57	SW0682T7	O	M
12	SW0142T7	O	O	58	SW0684	O	O
13	SW0162T7	M	N	59	SW0693T7	M	O
14	SW0181T7	O	O	60	SW0704M13	O	O
15	SW0184	M	O	61	SW0704T7	O	O
16	SW0208T7	O	O	62	SW0709M13	O	O
17	SW0212M13	O	O	63	SW0709T7	O	O
18	SW0212T7	O	O	64	SW0730T7	O	O
19	SW0249	M	O	65	SW0749T7	O	O
20	SW0277	O	O	66	SW0758T7	M	O
21	SW0292	O	O	67	SW0766	O	O
22	SW0305T7	M	O	68	SW0796M13	M	O
23	SW0306	O	O	69	SW0797T7	O	O
24	SW0328	M	O	70	SW0799T7	O	O
25	SW0337	O	O	71	SW0800T7	M	O
26	SW0345	O	O	72	SW0815T7	M	O
27	SW0348	M	O	73	SW0824M13	N	O
28	SW0353	O	O	74	SW0824T7	N	O
29	SW0389T7	O	O	75	SW0837	O	O
30	SW0392T7	M	O	76	SW0843T7	N	O
31	SW0402T7	O	O	77	SW0852	M	O
32	SW0410T7	M	O	78	SW0906T7	O	O
33	SW0411T7	M	M	79	SW0925	N	O
34	SW0433	O	O	80	SW0926T7	O	O
35	SW0445T7	O	O	81	SW0931T7	M	O
36	SW0450T7	O	M	82	SW0932	M	O
37	SW0464	O	O	83	SW0961T7	O	N
38	SW0466	M	O	84	SW0962	O	O
39	SW0469T7	M	O	85	SW0971	O	O
40	SW0489T7	O	O	86	SW0973T7	M	M
41	SW0498	O	O	87	SW0985	O	O
42	SW0511M13	O	O	88	SW1000M13	O	O
43	SW0511T7	O	O	89	SW1000T7	O	O
44	SW0519T7	O	M	90	SW1015T7	O	O
45	SW0522	O	O	91	SW1032T7	O	O
46	SW0539	O	O	92	SW1051	O	O

SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes	SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes
93	SW1052	O	O	142	SW0082T7	O	O
94	SW1053	O	O	143	SW0091T7	O	O
95	SW1059T7	O	O	144	SW0093T7	O	O
96	SW1067	M	O	145	SW0101M13	O	O
97	SW1068M13	O	O	146	SW0101T7	O	O
98	SW1068T7	O	O	147	SW0102T7	O	O
99	SW1085T7	M	O	148	SW0105T7	O	O
100	SW1086M13	M	O	149	SW0108T7	O	M
101	SW1086T7	M	O	150	SW0111T7	O	O
102	SW1088M13	O	O	151	SW0112T7	O	O
103	SW1088T7	O	O	152	SW0117T7	O	O
104	SW1089M13	O	O	153	SW0119T7	O	O
105	SW1089T7	O	O	154	SW0122T7	M	O
106	SW1093T7	O	O	155	SW0131T7	O	O
107	SW1098	O	O	156	SW0132T7	O	O
108	SW1115	O	O	157	SW0144T7	M	O
109	SW1116M13	O	O	158	SW0146T7	M	O
110	SW1116T7	O	O	159	SW0156T7	O	O
111	SW1122	O	O	160	SW0160T7	O	O
112	SW1138M13	O	O	161	SW0163T7	O	O
113	SW1138T7	O	O	162	SW0166T7	O	O
114	SW1139M13	O	O	163	SW0175T7	M	O
115	SW1139T7	O	O	164	SW0177M13	O	O
116	SW1144M13	O	O	165	SW0182T7	O	O
117	SW1144T7	O	O	166	SW0185T7	O	O
118	SW1145M13	M	O	167	SW0189T7	O	O
119	SW1187T7	O	O	168	SW0191T7	O	O
120	SW1195M13	M	O	169	SW0195T7	O	O
121	SW1195T7	M	O	170	SW0202T7	O	O
122	SW1209T7	M	N	171	SW0203T7	O	O
123	SW1225M13	O	O	172	SW0213T7	O	N
124	SW1225T7	O	O	173	SW0224T7	O	O
125	SW1227M13	M	O	174	SW0229T7	O	O
126	SW1227T7	M	O	175	SW0231M13	O	O
127	SW1242	M	O	176	SW0241T7	O	O
128	SW0004M13	O	O	177	SW0242T7	O	O
129	SW0004T7	O	O	178	SW0246T7	O	O
130	SW0011M13	O	O	179	SW0248T7	O	O
131	SW0011T7	O	O	180	SW0254T7	O	O
132	SW0015T7	O	O	181	SW0260T7	M	M
133	SW0024T7	M	O	182	SW0264T7	O	O
134	SW0026M13	O	O	183	SW0267T7	M	O
135	SW0026T7	O	O	184	SW0269T7	O	O
136	SW0033T7	O	O	185	SW0271T7	O	O
137	SW0038T7	M	O	186	SW0273T7	O	O
138	SW0069T7	O	O	187	SW0280T7	O	O
139	SW0073T7	O	O	188	SW0281T7	O	O
140	SW0076T7	O	O	189	SW0291T7	O	O
141	SW0078T7	O	O	190	SW0294T7	O	O

SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes	SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes
191	SW0295T7	O	O	240	SW0575T7	O	O
192	SW0296T7	O	O	241	SW0577T7	O	O
193	SW0297T7	O	O	242	SW0583T7	O	O
194	SW0301T7	O	O	243	SW0604T7	O	O
195	SW0310T7	O	O	244	SW0605M13	O	O
196	SW0311M13	O	O	245	SW0609T7	M	O
197	SW0325T7	O	O	246	SW0610M13	M	O
198	SW0326T7	O	O	247	SW0610T7	M	O
199	SW0330T7	M	O	248	SW0613T7	O	M
200	SW0334T7	O	N	249	SW0621T7	O	O
201	SW0339T7	O	O	250	SW0633T7	O	O
202	SW0341T7	O	O	251	SW0647T7	O	O
203	SW0358T7	O	O	252	SW0654M13	M	O
204	SW0359T7	M	O	253	SW0658T7	M	O
205	SW0360T7	O	O	254	SW0662T7	O	O
206	SW0361M13	O	O	255	SW0663M13	M	O
207	SW0367T7	O	O	256	SW0668T7	O	O
208	SW0369T7	O	O	257	SW0672T7	O	O
209	SW0394T7	O	O	258	SW0674T7	O	N
210	SW0399T7	O	O	259	SW0676T7	O	M
211	SW0401T7	O	O	260	SW0677T7	O	O
212	SW0403T7	O	O	261	SW0678M13	O	O
213	SW0412T7	M	O	262	SW0681T7	O	M
214	SW0419T7	O	O	263	SW0683T7	O	M
215	SW0429T7	M	M	264	SW0687T7	O	M
216	SW0434T7	O	O	265	SW0688T7	O	O
217	SW0441T7	O	O	266	SW0692T7	O	N
218	SW0446T7	O	O	267	SW0694T7	O	O
219	SW0454T7	O	O	268	SW0697T7	O	O
220	SW0461T7	O	O	269	SW0710T7	O	O
221	SW0468T7	O	O	270	SW0711T7	O	O
222	SW0484T7	O	U	271	SW0713T7	N	M
223	SW0489M13	O	U	272	SW0724T7	M	U
224	SW0496T7	O	U	273	SW0734T7	M	O
225	SW0499T7	O	O	274	SW0736T7	N	M
226	SW0507T7	O	M	275	SW0744T7	O	O
227	SW0514T7	O	M	276	SW0751T7	O	O
228	SW0520T7	O	M	277	SW0753T7	O	O
229	SW0531T7	M	N	278	SW0763T7	O	O
230	SW0537T7	M	N	279	SW0768T7	M	M
231	SW0548T7	O	U	280	SW0770T7	O	M
232	SW0555T7	O	N	281	SW0772T7	O	N
233	SW0557T7	O	N	282	SW0774T7	M	O
234	SW0560T7	O	N	283	SW0778T7	M	M
235	SW0563T7	O	U	284	SW0779T7	M	M
236	SW0570T7	O	O	285	SW0783T7	O	O
237	SW0572T7	O	M	286	SW0784T7	O	M
238	SW0573T7	M	U	287	SW0786T7	N	O
239	SW0574T7	O	O	288	SW0787T7	O	N

SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes	SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes
289	SW0797M13	O	O	338	SW1065T7	O	O
290	SW0803T7	O	O	339	SW1080T7	M	M
291	SW0809T7	O	N	340	SW1085M13	M	O
292	SW0811T7	M	N	341	SW1087T7	O	O
293	SW0815M13	M	O	342	SW1091T7	O	O
294	SW0821T7	O	O	343	SW1093M13	O	O
295	SW0825T7	M	M	344	SW1097T7	O	O
296	SW0826T7	M	M	345	SW1104T7	O	O
297	SW0827M13	O	O	346	SW1105T7	O	O
298	SW0828T7	O	M	347	SW1106T7	O	O
299	SW0836T7	M	O	348	SW1107T7	O	O
300	SW0839T7	O	M	349	SW1108T7	O	O
301	SW0843M13	N	O	350	SW1109T7	O	O
302	SW0846M13	O	M	351	SW1114T7	O	O
303	SW0847T7	O	M	352	SW1123T7	O	O
304	SW0849T7	M	M	353	SW1124T7	O	O
305	SW0850T7	O	O	354	SW1130T7	M	O
306	SW0855T7	O	O	355	SW1131T7	M	O
307	SW0863T7	M	M	356	SW1132T7	M	O
308	SW0866T7	O	O	357	SW1133M13	M	O
309	SW0867T7	N	O	358	SW1134T7	O	O
310	SW0896M13	N	O	359	SW1136T7	O	N
311	SW0912T7	O	O	360	SW1141T7	M	O
312	SW0914T7	O	O	361	SW1146T7	M	O
313	SW0916T7	O	O	362	SW1147T7	O	O
314	SW0918T7	O	O	363	SW1155T7	O	N
315	SW0921T7	N	O	364	SW1156T7	O	N
316	SW0923T7	O	O	365	SW1160T7	O	N
317	SW0926M13	O	O	366	SW1161T7	O	N
318	SW0928T7	N	M	367	SW1169T7	O	N
319	SW0947T7	O	O	368	SW1176T7	O	O
320	SW0949T7	O	O	369	SW1182T7	O	O
321	SW0954T7	M	O	370	SW1193T7	O	O
322	SW0964T7	M	N	371	SW1201T7	O	O
323	SW0969T7	M	N	372	SW1203T7	O	O
324	SW0972T7	M	N	373	SW1212T7	O	M
325	SW0982T7	O	M	374	SW1213M13	O	M
326	SW0994T7	O	N	375	SW1214T7	O	N
327	SW0998T7	O	N	376	SW1218T7	O	N
328	SW1001T7	O	O	377	SW1220T7	O	N
329	SW1002T7	O	N	378	SW1232T7	O	N
330	SW1012T7	O	O	379	SW1236M13	O	N
331	SW1018T7	O	M	380	SW1238T7	O	O
332	SW1045T7	O	M	381	SW1239T7	O	O
333	SW1046T7	M	O	382	SW1245M13	M	N
334	SW1058T7	O	O	383	SW1247T7	O	O
335	SW1059M13	O	O	384	SW0003T7	O	O
336	SW1061T7	O	O	385	SW0009T7	O	O
337	SW1064T7	O	O	386	SW0012T7	O	O

SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes	SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes
387	SW0013T7	O	O	436	SW0158T7	O	O
388	SW0015T7	O	O	437	SW0159T7	O	O
389	SW0016T7	U	N	438	SW0169T7	O	O
390	SW0018T7	O	O	439	SW0170T7	O	O
391	SW0019T7	O	O	440	SW0171T7	O	O
392	SW0023T7	O	O	441	SW0173T7	O	O
393	SW0025T7	O	O	442	SW0178T7	O	O
394	SW0027T7	O	O	443	SW0179T7	O	O
395	SW0029M13	O	O	444	SW0180T7	O	O
396	SW0030T7	O	O	445	SW0183T7	O	N
397	SW0039T7	O	O	446	SW0186T7	M	M
398	SW0043T7	O	O	447	SW0187T7	M	U
399	SW0046T7	O	O	448	SW0188T7	O	O
400	SW0048T7	O	O	449	SW0190T7	O	O
401	SW0050T7	O	O	450	SW0192T7	O	O
402	SW0052T7	O	O	451	SW0196T7	O	O
403	SW0063T7	O	O	452	SW0199T7	O	O
404	SW0064T7	O	O	453	SW0201T7	O	M
405	SW0068T7	O	N	454	SW0204T7	O	M
406	SW0072T7	O	O	455	SW0205T7	O	N
407	SW0074T7	O	N	456	SW0206T7	O	O
408	SW0075T7	O	O	457	SW0207T7	O	M
409	SW0077T7	O	O	458	SW0210T7	O	O
410	SW0080T7	O	O	459	SW0211T7	O	O
411	SW0081T7	O	O	460	SW0214T7	O	O
412	SW0085T7	O	O	461	SW0217T7	O	O
413	SW0088T7	O	O	462	SW0218T7	O	O
414	SW0090T7	O	O	463	SW0220T7	O	O
415	SW0095T7	O	O	464	SW0223T7	O	O
416	SW0103T7	M	O	465	SW0229T7	O	O
417	SW0104T7	M	O	466	SW0237T7	O	O
418	SW0121T7	O	N	467	SW0244T7	O	O
419	SW0123T7	O	O	468	SW0247T7	O	O
420	SW0125T7	O	O	469	SW0250T7	O	O
421	SW0127T7	O	O	470	SW0251T7	O	O
422	SW0128T7	O	O	471	SW0252T7	O	O
423	SW0129T7	O	O	472	SW0253T7	O	O
424	SW0130T7	O	N	473	SW0255T7	O	O
425	SW0133T7	M	M	474	SW0256T7	O	O
426	SW0134T7	O	O	475	SW0257T7	O	O
427	SW0135T7	M	O	476	SW0258T7	O	O
428	SW0140T7	O	O	477	SW0262T7	O	O
429	SW0141T7	M	O	478	SW0275T7	O	O
430	SW0143T7	O	O	479	SW0278T7	M	O
431	SW0145T7	O	O	480	SW0285T7	O	O
432	SW0147T7	O	O	481	SW0289T7	O	M
433	SW0152T7	O	O	482	SW0290T7	O	O
434	SW0155T7	O	N	483	SW0293T7	O	O
435	SW0157T7	O	O	484	SW0300T7	O	O

SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes	SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes
485	SW0302T7	O	O	534	SW0430T7	M	O
486	SW0303T7	O	O	535	SW0435T7	O	O
487	SW0307T7	O	O	536	SW0436T7	O	O
488	SW0308T7	O	O	537	SW0438T7	O	O
489	SW0311T7	O	O	538	SW0439M13	O	O
490	SW0312T7	O	O	539	SW0440T7	O	O
491	SW0313T7	O	O	540	SW0442M13	O	N
492	SW0314T7	O	O	541	SW0443T7	O	O
493	SW0319T7	O	O	542	SW0444T7	O	O
494	SW0322T7	O	N	543	SW0448T7	O	M
495	SW0333T7	O	O	544	SW0452M13	O	O
496	SW0338T7	M	O	545	SW0455T7	O	O
497	SW0340T7	O	O	546	SW0456T7	O	O
498	SW0342T7	O	O	547	SW0457T7	O	O
499	SW0344T7	O	O	548	SW0458T7	O	O
500	SW0346T7	O	O	549	SW0459T7	O	O
501	SW0347T7	O	O	550	SW0460T7	M	M
502	SW0349T7	M	O	551	SW0463T7	O	O
503	SW0350T7	O	O	552	SW0467M13	O	O
504	SW0351T7	O	O	553	SW0469M13	M	O
505	SW0352T7	O	O	554	SW0473M13	O	M
506	SW0354T7	O	O	555	SW0474T7	O	O
507	SW0355T7	O	O	556	SW0476T7	O	O
508	SW0356T7	O	M	557	SW0481T7	O	U
509	SW0357T7	O	O	558	SW0485T7	O	U
510	SW0361T7	O	O	559	SW0486T7	O	U
511	SW0362T7	O	O	560	SW0487T7	O	U
512	SW0365T7	O	O	561	SW0488T7	O	O
513	SW0366T7	O	O	562	SW0490T7	U	U
514	SW0381T7	O	O	563	SW0491T7	O	U
515	SW0391M13	O	O	564	SW0492T7	O	U
516	SW0393T7	O	O	565	SW0494T7	O	U
517	SW0395T7	O	M	566	SW0495T7	O	O
518	SW0396T7	M	O	567	SW0497T7	O	N
519	SW0398T7	O	O	568	SW0500T7	O	U
520	SW0400T7	O	O	569	SW0501T7	N or U	U
521	SW0404T7	O	O	570	SW0502T7	M	N
522	SW0405T7	O	O	571	SW0503T7	O	U
523	SW0406T7	M	O	572	SW0504T7	O	N
524	SW0407T7	O	O	573	SW0505T7	N	N
525	SW0408T7	M	O	574	SW0506T7	O	U
526	SW0413T7	M	O	575	SW0509T7	O	M
527	SW0414T7	O	U	576	SW0512T7	O	U
528	SW0415T7	O	O	577	SW0513T7	O	U
529	SW0417T7	N	O	578	SW0515T7	O	O
530	SW0418T7	O	O	579	SW0516T7	O	M
531	SW0426T7	O	O	580	SW0517T7	O	M
532	SW0427T7	O	O	581	SW0518T7	O	N
533	SW0428T7	M	U	582	SW0525T7	M	N

SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes	SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes
583	SW0529T7	O	N	632	SW0651T7	O	N
584	SW0532T7	O	N	633	SW0653T7	M	O
585	SW0533T7	O	N	634	SW0655T7	O	O
586	SW0534T7	O	M	635	SW0656T7	O	O
587	SW0535T7	O	O	636	SW0664T7	M	O
588	SW0536T7	M	U	637	SW0666T7	O	O
589	SW0538T7	O	N	638	SW0667T7	O	U
590	SW0540T7	O	O	639	SW0671T7	O	O
591	SW0541T7	O	O	640	SW0673T7	O	M
592	SW0542T7	O	O	641	SW0675T7	O	O
593	SW0543T7	O	O	642	SW0686T7	O	O
594	SW0544M13	O	M	643	SW0689T7	O	O
595	SW0545T7	O	O	644	SW0693M13	M	O
596	SW0546T7	O	O	645	SW0695T7	O	M
597	SW0547T7	O	U	646	SW0698T7	M	M
598	SW0550T7	O	M	647	SW0701T7	O	O
599	SW0551T7	O	M	648	SW0708T7	O	M
600	SW0552T7	O	U	649	SW0714T7	O	O
601	SW0554T7	O	U	650	SW0715T7	O	N
602	SW0559T7	O	M	651	SW0716T7	O	M
603	SW0561T7	O	N	652	SW0720T7	O	O
604	SW0562T7	O	U	653	SW0722T7	O	N
605	SW0566T7	O	O	654	SW0723T7	O	O
606	SW0567T7	O	N	655	SW0725T7	O	M
607	SW0568T7	O	N	656	SW0726T7	O	O
608	SW0569T7	O	O	657	SW0727T7	M	U
609	SW0571T7	O	O	658	SW0728T7	O	U
610	SW0578T7	O	N	659	SW0729T7	O	O
611	SW0580T7	O	O	660	SW0730M13	O	M
612	SW0582T7	O	O	661	SW0731T7	O	O
613	SW0584T7	O	O	662	SW0732T7	O	N
614	SW0591T7	N	O	663	SW0733T7	O	O
615	SW0606T7	O	O	664	SW0735T7	O	O
616	SW0607T7	O	O	665	SW0738T7	O	O
617	SW0608T7	O	O	666	SW0740T7	O	N
618	SW0611T7	O	O	667	SW0750T7	O	O
619	SW0612T7	N	O	668	SW0752T7	O	O
620	SW0616T7	O	M	669	SW0755T7	O	O
621	SW0623T7	O	O	670	SW0756T7	O	N
622	SW0629T7	O	O	671	SW0757T7	O	O
623	SW0635T7	O	O	672	SW0761T7	O	N
624	SW0636T7	O	O	673	SW0762T7	O	O
625	SW0637T7	O	M	674	SW0764T7	M	O
626	SW0640T7	N	O	675	SW0765T7	O	O
627	SW0641T7	O	M	676	SW0767T7	M	O
628	SW0642T7	O	O	677	SW0769T7	M	M
629	SW0644T7	O	O	678	SW0771T7	O	M
630	SW0645T7	O	O	679	SW0775T7	M	M
631	SW0646T7	O	O	680	SW0776T7	O	O

SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes	SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes
681	SW0780T7	O	O	730	SW0920T7	O	O
682	SW0782T7	M	M	731	SW0922T7	O	O
683	SW0785T7	O	O	732	SW0929T7	O	O
684	SW0789T7	O	O	733	SW0930T7	O	O
685	SW0790T7	O	N	734	SW0933T7	M	O
686	SW0795T7	O	O	735	SW0936T7	M	O
687	SW0796T7	M	M	736	SW0937T7	O	O
688	SW0798T7	M	M	737	SW0938T7	N	O
689	SW0799M13	O	O	738	SW0940T7	O	O
690	SW0801T7	O	O	739	SW0943T7	O	O
691	SW0802T7	M	M	740	SW0945T7	O	O
692	SW0804T7	O	O	741	SW0946T7	N	O
693	SW0806T7	O	M	742	SW0951T7	O	O
694	SW0807T7	N	N	743	SW0952T7	O	O
695	SW0810T7	M	O	744	SW0953T7	O	O
696	SW0814T7	O	O	745	SW0955T7	N	O
697	SW0816T7	N	N	746	SW0957T7	O	O
698	SW0819T7	O	O	747	SW0967T7	O	M
699	SW0822T7	O	M	748	SW0968T7	O	O
700	SW0827T7	O	O	749	SW0970T7	O	N
701	SW0829T7	O	M	750	SW0974T7	O	O
702	SW0830T7	O	M	751	SW0975T7	O	O
703	SW0831T7	O	O	752	SW0976T7	O	O
704	SW0834T7	O	O	753	SW0977T7	M	N
705	SW0835T7	O	N	754	SW0978T7	O	N
706	SW0838T7	O	U	755	SW0983T7	O	M
707	SW0840T7	O	O	756	SW0988T7	O	N
708	SW0842T7	O	O	757	SW0989T7	M	O
709	SW0845T7	O	O	758	SW0990T7	M	N
710	SW0846T7	O	M	759	SW0991T7	O	N
711	SW0848T7	O	M	760	SW0992T7	O	O
712	SW0851T7	M	M	761	SW0997T7	M	N
713	SW0853T7	O	O	762	SW1004T7	O	O
714	SW0854T7	N	O	763	SW1007T7	M	N
715	SW0857T7	O	O	764	SW1008T7	O	O
716	SW0858T7	M	N	765	SW1024T7	O	M
717	SW0859T7	M	M	766	SW1027T7	O	O
718	SW0860T7	O	M	767	SW1028T7	O	O
719	SW0862T7	M	M	768	SW1029T7	O	M
720	SW0865T7	N	O	769	SW1030T7	M	O
721	SW0868T7	O	O	770	SW1032M13	O	O
722	SW0891T7	O	O	771	SW1036T7	O	N
723	SW0897T7	O	O	772	SW1037T7	O	N
724	SW0898T7	O	O	773	SW1039T7	O	N
725	SW0901T7	O	O	774	SW1047T7	M	N
726	SW0904T7	O	O	775	SW1048T7	O	O
727	SW0905T7	N	O	776	SW1050T7	O	O
728	SW0917T7	O	O	777	SW1055T7	O	N
729	SW0919T7	O	O	778	SW1062T7	O	O

SEQ ID NO	clone name	Cell line probe	Cancer Tissue Probes
779	SW1063T7	O	O
780	SW1066T7	O	O
781	SW1069T7	O	O
782	SW1070T7	M	O
783	SW1074T7	O	O
784	SW1075T7	O	O
785	SW1076T7	O	O
786	SW1077T7	O	O
787	SW1078T7	O	O
788	SW1081T7	O	O
789	SW1082T7	O	O
790	SW1094T7	O	O
791	SW1095T7	O	N
792	SW1096T7	O	O
793	SW1099T7	O	O
794	SW1101T7	O	O
795	SW1103T7	O	O
796	SW1111T7	O	O
797	SW1112T7	O	O
798	SW1113T7	O	O
799	SW1117T7	O	O
800	SW1118T7	O	O
801	SW1119T7	O	O
802	SW1121T7	O	N
803	SW1125T7	O	O
804	SW1128T7	M	N
805	SW1129T7	O	O
806	SW1140T7	M	N
807	SW1143T7	O	O
808	SW1145T7	M	O
809	SW1149T7	M	O
810	SW1153T7	O	N
811	SW1157T7	O	O
812	SW1158T7	O	N
813	SW1164T7	O	M
814	SW1165T7	O	N
815	SW1166T7	O	O
816	SW1167T7	O	N
817	SW1170T7	M	N
818	SW1171T7	O	N
819	SW1172T7	O	N
820	SW1173T7	O	N
821	SW1175T7	O	N
822	SW1178T7	O	O
823	SW1179T7	O	O
824	SW1180T7	M	N
825	SW1183T7	O	M
826	SW1187M13	O	N
827	SW1189T7	O	N
828	SW1192T7	O	N
829	SW1196T7	M	N
830	SW1199T7	M	O
831	SW1200T7	O	M
832	SW1202T7	O	N
833	SW1204T7	O	N
834	SW1205T7	O	N
835	SW1207T7	O	N
836	SW1210T7	M	N
837	SW1213T7	O	M
838	SW1221T7	O	N
839	SW1223T7	O	O
840	SW1224T7	O	N
841	SW1228T7	O	O
842	SW1230T7	O	N
843	SW1231T7	O	O
844	SW1234T7	O	O
845	SW1235T7	O	N
846	SW1237T7	O	N
847	SW1240T7	O	O
848	SW1241T7	O	O
849	SW1243T7	O	O
850	SW1246T7	O	N

Table 2

SEQ ID NO	Clone name	"Novel" Region 1 Start / Stop	"Novel" Region 2 Start / Stop	GenBank Identifier for top 5 matching EST sequences				
128	SW0004M13	742-865		g1947473	g1969195	g2216795	g1236508	g1952906
129	SW0004T7	752-910		g1947473	g1969195	g2216795	g1236508	g2209605
130	SW0011M13	1-218	553-932	g2241970	g2140706	g1720731		
131	SW0011T7	1-264	599-890	g2241970	g2140706	g1720731		
132	SW0015T7	483-606		g675241	g900355	g706376	g1774265	g2337538
133	SW0024T7	1-148	268-606	g4033911	g1960000	g679294	g2180239	g942639
134	SW0026M13	400-598		g767139	g880785	g696474	g2558187	g2038504
135	SW0026T7	1-199	285-336	g767139	g880785	g696474	g2558187	g1494014
136	SW0033T7	427-610		g2873486	g1960450	g4440193	g2268964	g1721900
137	SW0038T7	321-645		g4222862	g2583432	g3052863	g2768420	g3229743
138	SW0069T7	366-612		g770924	g1308307	g4741105	g1844710	
139	SW0073T7	521-592		g1152099	g2191626	g1750705	g2025963	g1296011
140	SW0076T7	456-618		g2567157	g2236340	g2620190	g3754642	g2031668
142	SW0082T7	511-601		g1718668	g1274002	g2265780	g3214360	g1137129
146	SW0101T7	420-624		g1376510	g708780	g792817	g901666	g390100
147	SW0102T7	512-599		g4223023	g3430515	g3900153	g4125195	g2931421
148	SW0105T7	1-219	570-609	g2835475	g1482129	g1624179	g1817372	g2007732
149	SW0108T7	220-296	552-589	g2154028	g1303058	g1645371	g1792312	g2882934
150	SW0111T7	1-68		g1308307	g4332333			
153	SW0119T7	510-596		g4265953	g2836717	g4487239	g3228921	g2876545
154	SW0122T7	1-51		g1760809	g3804685	g2457104	g661521	
158	SW0146T7	1-76	333-617	g2009649	g985491	g1011403	g956142	g961346
159	SW0156T7	1-71	782-1002	g2902747	g3887935	g4223262	g4684438	g1162310
162	SW0166T7	1-48	444-638	g2264624	g3755582	g1891049	g4440147	g2357138
163	SW0175T7	1-303	829-1002	g724430	g2154572	g1958041		
166	SW0185T7	113-208		g1647210	g1647264	g3886862	g2444221	
168	SW0191T7	388-683		g829950	g771211	g766442	g2785582	g1441052
172	SW0213T7	449-617		g3886373	g955334	g1940943	g961389	g955941
174	SW0229T7	293-987		g2033455				

SEQ ID NO	Clone name	"Novel" Region 1 Start / Stop	"Novel" Region 2 Start / Stop	GenBank Identifier for top 5 matching EST sequences				
176	SW0241T7	494-570		g2010030	g2021290	g918739	g893980	g1976699
177	SW0242T7	1-41	440-621	g3645529	g4565156	g2335995	g1978587	g2019409
178	SW0246T7	1-202		g1162850	g1140707	g1990341	g1191239	g2538237
179	SW0248T7	497-650		g4079044	g2158663	g2788869	g1195625	g3750745
182	SW0264T7	1-94	479-609	g1976294	g3446793	g2459258	g1153656	g2577184
186	SW0273T7	1-89	546-638	g3677131	g3805522	g3244458	g4525163	g4598742
187	SW0280T7	412-628		g1815110	g1933167	g2817266		
188	SW0281T7	109-160	572-654	g2436919	g2185995	g3758001	g654599	g4523959
189	SW0291T7	461-650		g1992596	g1138351	g1146820	g395782	g1837320
190	SW0294T7	431-699		g2839339	g3838466	g1307860	g2617794	g1479221
196	SW0311M13	1-46	456-658	g4195712	g4648481	g2750125	g796654	g683242
197	SW0325T7	511-615		g1270394	g3896108	g2009344	g1238973	g2184702
198	SW0326T7	499-557		g1967113	g1967684	g1966134	g1966828	g2904744
200	SW0334T7	525-615		g1624696	g2356793	g1784223	g1774696	g1764577
202	SW0341T7	414-584		g774421	g570881	g1623681	g3040994	g1481791
203	SW0358T7	112-188	513-608	g1984379	g3789679	g3741829	g4531886	g1524800
204	SW0359T7	57-159	561-621	g1802072	g1663807	g1894318	g1775584	g1678033
206	SW0361M13	1-65	183-572	g2030884	g645753	g1988795	g1577434	g1578203
207	SW0367T7	559-616		g644105	g716356	g901097	g1188705	g712897
210	SW0399T7	486-589		g1856563	g1690249	g1966703	g1952828	g1639845
211	SW0401T7	470-590		g1165586	g1690123	g1967659	g1491055	g918845
212	SW0403T7	369-614		g3214476	g1648508	g1802846	g2703245	g1686573
213	SW0412T7	1-304	509-624	g681577	g712993	g4305548	g3428224	g318414
214	SW0419T7	134-612		g1388511	g4533033	g2552190	g3240798	g3366974
215	SW0429T7	516-618		g1349681	g1269881	g4522374	g1272714	g3933264
216	SW0434T7	349-595		g4261346	g3596444	g3755357	g3329909	g4684571
217	SW0441T7	428-610		g4762076	g2158733	g2158750	g2809783	g2113084
218	SW0446T7	458-585		g4111486	g1484542	g3415988	g1959348	g2874960
219	SW0454T7	116-599		g1319069	g1319055	g2669407	g2355953	g3181853
220	SW0461T7	1-189	411-602	g1295370	g2008512	g1783876	g1571056	
221	SW0468T7	1-55	477-573	g2163292	g2162568	g4534378	g1225564	g1696820
223	SW0489M13	449-564		g1779025	g2027299	g1960180	g2016248	g2879596